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(54) IMMUNOLOGICAL AGGLUTINATION REACTION REAGENT FOR DIAGNOSIS OF HEPATITIS C

(57)Abstract:
PURPOSE: To obtain an immnological agglutination reaction reagent for diagnosis of hepatitis C excellent in diagnosis sensitivity and specificity by employing hepatitis C virus(HCV) antigen active polypeptide of gene, derived from heat treated HCV, as an antigen.
CONSTITUTION: The immunological agglutination reaction reagent for diagnosis of HCV employs HCV antigen active polypeptide of gene, derived from heat treated HCV, as an antigen. The HCV antigen active polypeptide consists of HCV antigen active polypeptide including amino acid sequence No.1, HCV antigen active polypeptide including amino acid sequence No.2, and HCV antigen active polypeptide including amino acid sequence No.3, and HCV antigen active polypeptide including amino acid sequence of No.4, all of them being carried on insoluble carrier particles. Heat treatment of the mixture of polypeptide is carried out effectively at 20-80° C, preferably at 25-60° C. Heat treatment is carried out in a buffer.

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CLAIMS

[Claim(s)]
[Claim 1] The immunological agglutination reaction reagent for a hepatitis C diagnosis characterized by using the HCV antigen activity polypeptide of the heat-treated hepatitis C virus origin gene as an antigen.
[Claim 2] The immunological agglutination reaction reagent of claim 1 characterized by including a HCV antigen activity polypeptide including the amino acid sequence of the HCV antigen activity polypeptide in which a HCV antigen activity polypeptide includes the amino acid sequence of the array number 1, a HCV antigen activity polypeptide including the amino acid sequence of the array number 2, a HCV antigen activity polypeptide including the amino acid sequence of the array number 3, and the array number 4.
[Claim 3] The immunological agglutination reaction reagent for a hepatitis C diagnosis characterized by coming to support a HCV antigen activity polypeptide including the amino acid sequence of a HCV antigen activity polypeptide including the amino acid sequence of the array number 1, a HCV antigen activity polypeptide including the amino acid sequence of the array number 2, a HCV antigen activity polypeptide including the amino acid sequence of the array number 3, and the array number 4 to an insoluble support particle.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to the immunological agglutination reaction reagent for a hepatitis C diagnosis for detecting the antibody to the hepatitis C virus (it being hereafter written also as HCV) which is the cause of a disease of hepatitis C.
[0002]
[Description of the Prior Art] Hepatitis C is caused by HCV and 90 percent or more of the after [transfusion] un-A un-B mold chronic hepatitis is called hepatitis caused by infection of HCV. A part of gene of HCV is reported to European Patent EP0318216 (1989 public presentation) and European Patent EP0388232 (1990 public presentation).
[0003] According to old research, HCV is considered to be the RNA virus of about 10 gene sequence overall length kbs (about 10,000 nucleotides). The reagent for a hepatitis C diagnosis has been developed by using as an antigen a polypeptide with antigen activity high among the proteins produced from a HCV gene. For example, in the European Patent 0318216, the reagent for a hepatitis C diagnosis which used for the antigen the HCV antigen activity polypeptide which inserts in the expression vector of yeast a part of gene which carries out the code of the non-structure protein field, is made to discover this gene, and is called C100 is also one of them.
[0004] The detection method by the enzyme immunoassay (it abbreviates also to the EIA method hereafter) and passive agglutination (it abbreviates also to the PA method hereafter) which used current, one sort, or two sorts of HCV antigen activity polypeptides as an antigen is used for the hepatitis C diagnosis. The above-mentioned diagnostic drug is obtaining effectiveness for prevention of the hepatitis C at the time of transfusion. However, since one sort and two sorts of HCV Hara activity polypeptides are used as an antigen, the present condition is that there are much false negative and false positivity, and a problem is in the sensibility of the diagnosis in early stages of HCV infection.
[0005]
[Problem(s) to be Solved by the Invention] Although the hepatitis C diagnostic drug used now is obtaining big effectiveness for prevention of infection of the hepatitis C virus at the time of transfusion, development of a diagnostic drug diagnosable in early stages of HCV infection with more high and sensibility and singularity is desired. By using as an antigen the HCV antigen activity polypeptide of the hepatitis C virus gene origin which this invention person studied wholeheartedly that this technical problem should be solved, consequently was heated, it finds out that the immunological agglutination reaction reagent for a hepatitis C diagnosis which was excellent in the sensibility of a diagnosis and singularity is obtained, and came to complete this invention.
[0006]
[Means for Solving the Problem] This invention is in the immunological agglutination reaction reagent for a hepatitis C diagnosis characterized by using the HCV antigen activity polypeptide of the heat-treated hepatitis C virus origin gene as an antigen. The above-mentioned HCV antigen activity polypeptide contains a HCV antigen activity polypeptide including the amino acid sequence of the array number 1, a HCV antigen activity polypeptide including the amino acid sequence of the array number 2, a HCV antigen activity polypeptide including the amino acid sequence of the array number 3, and a HCV antigen activity polypeptide including the amino acid sequence of the array number 4.
[0007] Furthermore, this invention is in the immunological agglutination reaction reagent for a hepatitis C diagnosis which comes to support a HCV antigen activity polypeptide including the amino acid sequence of a HCV antigen activity polypeptide including the amino acid sequence of the array number 1, a HCV antigen activity polypeptide including the amino acid sequence of the array number 2, a HCV antigen activity polypeptide including the amino acid sequence of the array number 3, and the array number 4 to an insoluble support particle.

[0008] This invention is explained below at a detail. Heat-treatment in this invention is performed by heating the mixture of a polypeptide to predetermined temperature. Whenever [this stoving temperature] has 20 degrees C or more effective 80 degrees C or less. It is 25 degrees C or more 60 degrees C or less preferably, and is 35 degrees C or more 50 degrees C or less still more preferably. Heat-treatment of a polypeptide may be performed in the buffer solution with buffer action, and any are sufficient as the class. For example, a phosphate buffer, the glycine buffer solution, tris buffers, the acetic-acid buffer solution, etc. are **. Also about pH, although any are sufficient, a neutral region is desirable. desirable -- a phosphate buffer and pH6.0 from — 8.0 is desirable. If the processing time is more than for 10 minutes, any are sufficient as it and it is 2 or less hours more than for 30 minutes still more preferably from for 10 minutes preferably for 5 or less hours.
[0009] The hepatitis C virus gene said by this invention is RNA of about 10 overall length kbs (about 10,000 nucleotides) which has for example, Proc.Natl.Acad.Sci.USA, Vol.87, and the base sequence indicated by pp.9524 -9528 (1990). Although HCV is an RNA virus, cDNA made with reverse transcriptase from RNA of the HCV origin also corresponds to this hepatitis C virus gene.
[0010] This hepatitis C virus gene can be obtained from the cDNA library which separated and produced virogene from the blood serum of the non-A-non-B-hepatitis patient after transfusion. For example, ultracentrifuge separates a hepatitis C virus from patient's serum first, subsequently Gene RNA is prepared from a virus, cDNA is compounded to this RNA using reverse transcriptase, this cDNA fragment is inserted in the appropriate back at a plasmid vector or a phage vector, and a cDNA library is prepared. Subsequently, the target gene can be obtained by carrying out immuno screening of this cDNA library using the blood serum (blood serum containing an anti-HCV antibody) of the non-A-non-B-hepatitis patient after transfusion. Moreover, a DNA probe may be compounded based on the base sequence of a well-known HCV gene, and a cDNA library may be screened by DNA/DNA hybridization. Moreover, it is an option. The approach of making carry out gene amplification of the target field by RT-PCR method which combined the approach shown in Proceedings of the Japan Academy, Vol.65, Ser.B, No.9, pp.219 - 223(1989)., i.e., reverse transcriptase and the PCR method, and carrying out cloning of the gene fragment made to amplify is also effective.
[0011] The HCV antigen activity polypeptide in this invention has the anti-HCV antibody and immunological reactivity which are included in hepatitis C patient's serum and plasma. That is, it has an epitope part to an anti-HCV antibody, and has the property specifically combined with the anti-HCV antibody in hepatitis C patient's serum and plasma by the antigen-antibody reaction. This HCV antigen activity polypeptide can be used as an antigen of the reagent for a hepatitis C diagnosis.
[0012] This HCV antigen activity polypeptide is a polypeptide produced with the gene of HCV. Any are sufficient as the die length of a HCV antigen activity polypeptide, and, furthermore, it is 3 or more amino acid residue 2000 or less amino acid residue 3 or more amino acid residue 3000 or less amino acid residue still more preferably preferably. This HCV antigen activity polypeptide can be discovered using the gene expression system usually known, i.e., the host vector system of Escherichia coli, the host vector system of a Bacillus subtilis, the host vector system of yeast, an insect cell or the host vector system of an insect, the host vector system of an animal cell, etc. Among these, Escherichia coli can be used suitably. In order to discover this HCV antigen activity polypeptide using Escherichia coli, the gene of HCV is inserted in the vector which can be first discovered with Escherichia coli, and a recombination vector is produced. Especially a vector is not limited, but although any vectors can be used if it is the vector usually used as a vector of Escherichia coli, the vector to which especially gene expression happens by high frequency is used suitably. For example, a series of pUC vectors (TAKARA SHUZO CO., LTD. product), a series of pTV vectors (TAKARA SHUZO CO., LTD. product), a series of pTZ vectors (Toyobo Co., Ltd. product), a series of pET(s) (shown in Methods in enzymology and Vol.185), etc. can be used. Moreover, if a series of pUEX vectors (Amersham Japan product) and a series of pEX vectors (Boehringer Mannheim Yamanouchi product) are used, a HCV antigen activity polypeptide can be made to discover as a fusion polypeptide with the beta-galactosidase. The promotor for the gene expression usually committed within Escherichia coli and the operator who controls it are attached to the vector which can be discovered with Escherichia coli. A recombination vector is produced by inserting a HCV gene using the suitable restriction enzyme part on the lower stream of a river of the promotor of such a vector. The transformation of the Escherichia coli is carried out by the recombination vector, and a HCV antigen activity polypeptide is produced by making the HCV gene which cultivated this transformation Escherichia coli and was inserted discover.
[0013] When performing gene expression by the recombination vector, two or more amino acid of a random array may add to the amino terminal or C terminal of a polypeptide. However, since two or more amino acid added to such an amino terminal or the C terminal is random amino acid, it is unrelated to HCV antigen activity, and does not influence antigen activity measurement.
[0014] This HCV antigen activity polypeptide crushes the fungus body which may have had the above-mentioned

transformation Escherichia coli cultivated by approaches, such as sonication, and is separated from this fungus body debris by the well-known approach. If the purification approach of this HCV antigen activity polypeptide is a well-known approach, any are sufficient as it and they are **, such as a salting-out, ion-exchange-resin adsorption, and gel filtration. The combination of the above-mentioned approach is preferably effective. Moreover, although this refined HCV antigen activity polypeptide may be distributed by what kind of solution, it is desirable for 0.87% sodium chloride water solution (for it to be hereafter written also as a physiological saline) or 0.87% sodium chloride content, 20mM phosphate buffer, and pH7.2 (for it to be hereafter written also as PBS) to distribute preferably. Moreover, the higher one as purification purity is good. This HCV antigen activity polypeptide is preferably [80% or more in the quality of total protein of] desirable.

[0015] The 1st HCV antigen activity polypeptide as used in the field of this invention is a polypeptide (it is hereafter written also as a Core antigen) which includes the amino acid sequence shown in the indispensable array number 1 in the outstanding antigen activity, and two or more excessive amino acid may add to the amino terminal or C terminal. This array is equivalent to the amino acid sequence of the core protein from the amino terminal of the Japanese mold HCV to the 1st thru/or the 168th.

[0016] The 2nd HCV antigen activity polypeptide as used in the field of this invention is a polypeptide (it is hereafter written also as NS-3 antigen) which includes the amino acid sequence shown in the indispensable array number 2 in the outstanding antigen activity, and two or more excessive amino acid may add to the amino terminal or C terminal. The 1st to the 211st of this array counts from the amino terminal of the Japanese mold HCV, and they are equivalent to the amino acid of NS-3 protein from the 1323rd to the 1533rd.

[0017] The 3rd HCV antigen activity polypeptide as used in the field of this invention is a polypeptide (it is hereafter written also as NS-4 antigen) which includes the amino acid sequence shown in the indispensable array number 3 in the outstanding antigen activity, and two or more excessive amino acid may add to the amino terminal or C terminal. The 1st to the 194th of this array counts from the amino terminal of the Japanese mold HCV, and they are equivalent to the amino acid of NS-4 protein from the 1605th to the 1798th.

[0018] The 4th HCV antigen activity polypeptide as used in the field of this invention is a polypeptide (it is hereafter written also as NS-5 antigen) which includes the amino acid sequence shown in the indispensable array number 4 in the outstanding antigen activity, and two or more excessive amino acid may add to the amino terminal or C terminal. The 1st to the 160th of this array counts from the amino terminal of the Japanese mold HCV, and they are equivalent to the amino acid of NS-5 protein from the 2111st to the 2270th.

[0019] If it is the support which can be used for the diagnostic drug of a condensation method well-known as an insoluble support particle in this invention, anything, it is good, for example, they are the high-specific-gravity composite particle (it is written also as HDP JP,62-115366,A and the following) which made the minerals compound used as hof cover a color, a sheep erythrocyte, a polystyrene particle, a gelatin particle, etc. HDP, a sheep erythrocyte, and polystyrene are used preferably. It is HDP still more preferably. Moreover, if the particle diameter of the insoluble support used by this invention is also the thing of the range to use as a condensation method diagnostic reagent, any are sufficient, and it is the thing of the particle diameter from 0.01 micrometers to 20 micrometers preferably, and is a 0.01 to 3 micrometers thing still more preferably. Moreover, which thing is sufficient also as the specific gravity of insoluble support, and it is 1.0 to 2.5 preferably.

[0020] As long as the support as used in the field of this invention is the well-known approach by which it adsorbs by the approach of making a HCV antigen activity polypeptide sticking to insoluble support, any are sufficient and ******** is sufficient as a physical adsorption process, a chemical adsorption process, etc. For example, canal-adsorption, a chromium chloride method, etc. are **. A canal-adsorption process is preferably desirable. Said support may be performed in the buffer solution with buffer action, and any are sufficient as the class. For example, a phosphate buffer, the glycine buffer solution, tris buffers, the acetic-acid buffer solution, etc. are **. Also about pH, although any are sufficient, a neutral region is desirable. 8.0 is preferably desirable from a phosphate buffer and pH6.0.

[0021] When making support support a HCV antigen activity polypeptide, although there is especially no limitation in protein concentration, ml is preferably suitable for it to it more than in 0.1microg /. Moreover, although not limited to especially the time amount and temperature that an insoluble support particle is made to support, temperature is desirable, and 1-degree-C or more 80 degrees C or less and time amount are more than for 30 minutes, and can be performed suitably. Although the immunological agglutination reaction reagent for a hepatitis C diagnosis of this invention is used in the state of aqueous suspension, it is desirable to freeze-dry this in long-term preservation. The reagent for agglutination reactions of this invention shows the outstanding engine performance, without the piece of the stability at the time of the preservation again described above also as aqueous suspension and the condensation image of reaction time falling after this freeze drying. What is necessary is just to perform the above-mentioned freeze-drying approach by the usual approach which is not restrictive. For example, the approach and conditions which are adopted as the freeze drying method of a

sensitized erythrocyte are used. The approach of carrying out rapid preliminary freezing preferably and subsequently freeze-drying is adopted. In this rapid preliminary freezing, they are liquid nitrogen and dry ice. A methanol, dry ice It is attained by immersing containers, such as a vial or ampul with which the above-mentioned aqueous suspension went into an acetone or fluorocarbon.

[0022] Moreover, generally, after the freeze-drying approach carries out rapid preliminary freezing of the vial containing the suspension of the above-mentioned sensitization support etc., the approach of placing into the chamber of the freeze dryer beforehand cooled at -40--60 degree C, carrying out a temperature up gradually over 24 - 72 hours, and freeze-drying is suitable for it. 20-50 degrees C is suitable for the pressure Hg of 50-200micro and the last drying temperature in the chamber at this time. Subsequently, what is necessary is to be filled up with a vacua or inactivation gas and just to carry out sealing plug preservation. However, the freeze-drying approach is not limited to said approach.

[0023] The reagent for agglutination reactions of this invention is applied that the agglutination reaction method usually used for a diagnosis does not have a limit in any way. For example, they are the plate process of a qualitative diagnosis, the microtiter technique of a half-quantum diagnosis and the nephelometry of a quantum diagnosis, particle number mensuration, etc. Among those, when applying to especially a microtiter technique, especially the effectiveness of this invention is remarkable. The immunological agglutination reaction reagent for a hepatitis C diagnosis as used in the field of this invention is a reagent for a diagnosis which diagnoses hepatitis C by detecting the anti-HCV antibody which exists in a hepatitis C patient's blood serum, or plasma by the immunological agglutination reaction. Usually, the agglutination reaction method used for a diagnosis is applied that there is no limit in any way. For example, they are the plate process of a qualitative diagnosis, the microtiter technique of a half-quantum diagnosis and the nephelometry of a quantum diagnosis, particle number mensuration, etc. Among those, when applying to especially a microtiter technique, especially the effectiveness of this invention is remarkable.

[0024]

[Effect of the Invention] The immunological agglutination reaction reagent for a hepatitis C diagnosis of this invention is remarkably excellent in detection sensitivity and singularity compared with elegance conventionally, and can be judged in a short time. Moreover, the condensation formation object (it is hereafter written also as a sedimentation pattern) by the antigen-antibody reaction which is the criteria of a judgment of an agglutination reaction is formed very clearly. Therefore, the immunological agglutination reaction reagent for a hepatitis C diagnosis of this invention is a hepatitis C diagnostic reagent which was extremely excellent as compared with the conventional thing.

[0025]

[Example] An example and the example of a comparison are given to below, and this invention is explained to it still more concretely. However, the technical range of this invention is not limited by these examples. Especially in this example, unless it refused, the technique of a genetic manipulation experiment was performed according to Sam Brock's and others approach [Sambrook, J., Fritsch, E.F., Maniatis, T., Molecular Cloning, 2nd ed., Cold Spring Harbor Laboratory Press, and New York. (1989)]. In addition, the restriction enzyme used the TAKARA SHUZO CO., LTD. product.

[0026] (Example 1) Manufacture (1-1) recombination plasmid pGC03 of a Core antigen It is 19,000rpm in 3000ml of non-A-non-B-hepatitis patient's sera after production (preparation of RNA) transfusion. Ultracentrifuge was carried out for 16 hours and precipitation was obtained. these settlings -- 100ml (4M GUANIJIUMU isothiocyanate (product made from FURUKA), 25mM citric-acid soda, 0.5 % sarcosyl, 0.1M mercaptoethanol) of GITC solutions dissolving -- 100ml of these melts receiving -- 100ml Phenol-chloroform (1: 1) adding -- the room temperature during 15 minutes -- after shaking and 3000rpm -- the at-long-intervals alignment was carried out for 15 minutes. 3000rpm after taking out the water layer of this reaction mixture, adding isopropyl alcohol 100 ml and leaving it at -20 degrees C for 3 hours The at-long-intervals alignment was carried out for 15 minutes, and settlings were obtained.

[0027] To these settlings, 10ml of GITC solutions was added and it considered as the solution. It is 10ml phenol-chloroform to this solution. (1: 1) It is after shaking, 3000rpm, and 15 at a room temperature for 10 minutes moreover. The part at-long-intervals alignment was carried out. The water layer of this reaction mixture was taken out, chloroform 20ml was added, and it shook for 5 minutes. After shaking and 3000rpm The at-long-intervals alignment was carried out for 5 minutes, and 10ml of water layers was collected. It is 5M NaCl to 10ml of this water layer. Solution 0.4ml was added.

[0028] Then, 30ml ice-cooling ethanol was added and it was left at -20 degrees C for 12 hours. After neglect and 3000rpm The at-long-intervals alignment was carried out for 15 minutes, and settlings were obtained. These settlings are washed by ethanol 75%, and they are after desiccation and distilled water 200. mul It dissolved and the RNA solution was obtained.

(Construction of a cDNA library) cDNA composition used the synthetic kit of BRL. The approach was performed according to the cDNA composition manual [BRL/Cosmobio Instruction Manual, and Cat.No8267SA]. Single-stranded-RNA solution 5 prepared from un-A un-B patient's sera by the term of (preparation of RNA) of this example mul It is random primer solution (100microM) [the TAKARA SHUZO CO., LTD. product and the product catalog number 3810] 5 mul In addition, the reverse transcriptase reaction was performed and it considered as 2 chains of RNA/DNA. Subsequently, Escherichia coli DNA polymerase I and Escherichia coli ribonuclease H were added, and it considered as the DNA/two DNA chain.

[0029] Next, it is EcoRI to the both ends of the double stranded DNA obtained in this way. The linker was combined. It reacted to this processing by the reaction condition attached to the enzyme of TAKARA SHUZO using the enzyme of TAKARA SHUZO. It is the double stranded DNA 1 [about] first. mug It uses and is EcoRI. Methylase processing is performed and it is T four after that. It is EcoRI by the DNA ligase reaction. Linker (dGGAATTCC) It was made to join together. It is EcoRI about the reaction mixture obtained at the end. It cuts and is EcoRI. Fragments were collected.

[0030] It is this EcoRI to the last. It is EcoRI of lambdagt11 about a fragment. Although it inserted in the part, it rearranged and lambdagt11 phage was produced, in this, it is the kit GIGAPACKII GOLD of Stratagene. Using, the approach followed the manual [Protocol/Instruction Manual Cat.#200214, 200215, 200216, December 6, and 1989] attached to the kit. It is EcoRI of lambdagt11 first. It is EcoRI to a part. A fragment is inserted and it is this T four It was made to join together with a DNA ligase. It is GIGAPACKII GOLD about the obtained recombination phage DNA solution. It returned to phage using In Vitro Packaging Kit. the place which titrated the titer at this time -- 1.0x106 it was . This titer value shows the number of the independent clones.

(Immuno screening) If the frame of cDNA inserted in lambdagt11 corresponds, the amino acid sequence cDNA is carrying out [the amino acid sequence] the code will be expressed as a fusion protein with the beta-galactosidase included in lambdagt11. It is what absorbed non-A-non-B-hepatitis patient's serum by the fungus body of Escherichia coli, and this fusion protein was screened. The indicator bacterium used E.coli Y1090. The phage liquid and Y1090 which were prepared so that a plaque might become about 40,000 pieces per plate at L-bottom plate (Bacto-tryptone 10g, NaCl 5g, Yeastextract 5g, and Bacto-agar 15g are added per water 1 liter, and it is autoclave sterilization) with a diameter of 15cm It incubated at 37 degrees C for 15 minutes. 0.7 %L-top agarose 2.5ml warmed at 45 degrees C at it It mixes, extends to L-bottom plate, and is 3.5 at 42 degrees C after solidification. Time amount incubation was carried out. On the other hand, it is 10mM isopropyl thio-beta-D about a nitrocellulose filter. After dipping in - galactoside (IPTG) solution for several minutes, it dried at the room temperature. This filter was put on the top this plate, and overnight incubation was carried out at 37 degrees C. The filter was stripped after incubation, it dipped in the TNT buffer solution (10mM tris - HCl (pH8.0), 150mM NaCl, and 0.05% Tween20), and the rinse was improved. It dipped for 30 minutes, shaking to the new TNT buffer solution again. Furthermore, this filter was incubated for 30 minutes with the blocking buffer solution (20% fetal-calf-serum content TNT buffer solution). Next, it is a filter with the blocking buffer solution 150 It was made to react, shaking slowly for 4 hours at the primary antibody liquid (what absorbed the non-A-non-B-hepatitis patient pooled serum with the ultrasonic crushing liquid of Y1090) and the room temperature which were double-diluted. subsequently, a filter -- the 0.1 % cow serum albumin (BSA) content TNT buffer solution and 0.1 %BSA+0.1 %NP-40 It washed every [during 10 minutes] in order of the content TNT buffer solution and the 0.1 %BSA content TNT buffer solution. the next -- 10microl The 0.1 after dipping filter in the 15ml blocking buffer solution containing horseradish peroxidase indicator anti-Homo sapiens IgG goat IgG (product made from Kirkeguard & Perry Lab) and making it react at room temperature for 2 hours %BSA content TNT buffer solution, and 0.1 %BSA+0.1 %NP-40 The content TNT buffer solution washed every [during 10 minutes]. Furthermore, it is 10mM tris about a filter. - By HCl (pH7.5) and 150mM NaCl, after washing during 1 minute, Methanol 20ml containing a stain solution [60mg 4-chloro-naphthol just before use 30%H2O2 60microl Included 10mM tris - It reacted to thing] mixed with HCl (pH7.5) and 100ml of 150mM NaCl solutions at the room temperature for 15 minutes, and after distilled water washed twice, the electropositive plaque colored purple was obtained.

[0031] Phage DNA is prepared from this recombination phage, and it is EcoRI. It processes, the fragments of cDNA are collected from agarose electrophoresis gel, and it is a plasmid vector pUC18. EcoRI It inserted in the part. It is this plasmid pGC03 It named and the base sequence was determined. That the core region of the structural protein gene of HCV is contained in this cDNA fragment became whether to be **.

(1-2) Escherichia coli HB one 101 Production pGC03 of [pHCX01] HinfI The end was graduated by DNA polymerase I Klenow fragment after digestion. This DNA and BamHI It is a linker (dCGGATCCG, TAKARA SHUZO CO., LTD. make) T four A DNA ligase performs ligation and it is BamHI further. It digested and the 0.56kb fragments containing a core region were collected from agarose electrophoresis gel. It is a plasmid vector pUC19 about this 0.56kb fragment. BamHI It inserts in a part and is this plasmid at BspHI (New England Biolabs shrine product) further After digestion and T four The end was graduated by DNA polymerase. It is BamHI about

this DNA. It digested and the core-region DNA fragments of 0.51kbs except 5' side untranslation region were collected from agarose electrophoresis gel. It is this 0.51kb fragment SmaI-BamHI of a plasmid vector pUEX2 (product made from Amersham) It inserted in the part and the recombination vector pHCX01 was obtained. About obtained pHCX01, the approach of decision [Hattori and others of the base sequence by the plasmid method, Anal.Biochem., Vol.152, and pp.232- 238 (1986) were performed. The base sequence which carries out the code of the 1st thru/or the 168th amino acid sequence from the amino terminal of HCV is included in this recombination vector pHCX01, and that base sequence is as being shown in the array number 5. Next, it is host Escherichia coli HB one 101 at the recombination vector pHCX01. A transformation is carried out and it is recombination Escherichia coli HB one 101. [pHCX01] was obtained. Recombination Escherichia coli HB one 101 [pHCX01] is deposited with the Fermentation Research Institute of 1-1-3, Higashi, Tsukuba-shi, Ibaraki-ken, the Agency of Industrial Science and Technology, the Ministry of International Trade and Industry, as a Fermentation Research Institute mycoparasite No. 13056. This recombination Escherichia coli HB one 101 [pHCX01] was cultivated at 30 degrees C overnight by the LB+Amp culture medium [Bacto tryptone 1.0%, Yeast extract 0.5%, NaCl0.5%, ampicillin (Amp) 50 mug / ml], the glycerol was added and cryopreservation was carried out at -80 degrees C so that the last concentration might become 15%.

(1-3) Manufacture recombination Escherichia coli HB one 101 of a Core antigen By cultivating [pHCX01] and performing gene expression, a Core antigen is produced as a fusion polypeptide with the beta-galactosidase. Recombination Escherichia coli HB one 101 1ml of cryopreservation fungus bodies of [pHCX01] was inoculated into the 1l. LB+Amp culture medium, and they were cultivated at 30 degrees C overnight. Then, inoculation of this culture is carried out to a 20l. LB+Amp culture medium, and it is OD540 at 30 degrees C. 1.5 It cultivated until it became, and 42 degrees C was raised and culture temperature was cultivated succeedingly for 3 hours. A harvest is carried out after culture and according to centrifugal separation, and it is 57g. The wet fungus body was obtained. TNE which contains 0.6M 2l. urea for a fungus body It suspended in the buffer solution (50mM Tris and HCl (pH8.3), 100mM NaCl, 1mM EDTA), and crushed by sonication. The insoluble granulation which contains a Core antigen for this fungus body debris according to 10,000g and the centrifugal separation for 20 minutes was collected to the precipitation fraction. TNE which includes this precipitation for 0.6M 2l. urea again It suspended in the buffer solution and precipitation was collected by washing and carrying out centrifugal separation of the insoluble granulation. Furthermore, TNE which includes this precipitation for 3M 2l. urea It suspended in the buffer solution, and after washing insoluble granulation enough by stirring for 30 minutes at a room temperature, insoluble granulation was collected to the precipitation fraction by carrying out centrifugal separation. To precipitation of this insoluble granulation, it is 200ml. The TNE buffer solution containing 8M urea was added, and precipitation was solubilized. Supernatant liquid is isolated preparatively for this according to 16,000g and the centrifugal separation for 20 minutes, and it is TNE. It dialyzed to the buffer solution. After dialysis, supernatant liquid was isolated preparatively according to 16,000g and the centrifugal separation for 20 minutes, and the Core antigen was obtained. 20l. culture medium to 980mg The Core antigen was obtained. It checked that it was in agreement with the molecular weight (137kd) which investigates molecular weight by SDS polyacrylamide electrophoresis (SDS-PAGE), and is calculated from the amino acid sequence about the obtained Core antigen.

[0032] (Example 2) Manufacture (2-1) recombination plasmid pHCV7 of NS-3 antigen Gene amplification by RT-PCR method was performed about the gene fragment of NS3 field of HCV where it is expected that production ****** antigen activity is shown, using the primer of 20 every bases of both sides of the fragment as a set. The primer was compounded using the applied biotechnology systems company product and 340A mold machine. In addition, the base sequence of CCGACGGTGGATGCTCCGG(5') G (3') and 3' downstream primer of the base sequence of 5' upstream primer is CTGGAGCCAATCCAACGCC(5') C (3').

[0033] First, RNA solution 4 obtained in the example 1 mul Reverse transcriptase reaction mixture [250mM Tris and HCl (pH8.3), 375mM KCl, 50mM DTT, and 15mM MgCl 2] 2 mul, antisense strand primer solution (25ng/mul) 1 of 3' downstream mul and four kinds of deoxy nucleotides [dATP, dGTP, dCTP, dTTP, 15 mM(s) each] -- each 0.5 mul every -- 9 [in addition,] mul The solution was made. A mineral oil is added to this, and 70 degrees C is heated for 2 minutes, and subsequently to 37 degrees C it cools, and is reverse transcriptase 1. mul (BRL product) was added and it was made to react at 37 degrees C for 60 minutes. this reaction mixture (10 mul) -- further -- PCR reaction mixture [400mM Tris and HCl (pH8.8), a 100mM ammonium sulfate, a 40mM magnesium chloride, 60mM mercaptoethanol, and 0.1 % BSA] 8.3 mul and four kinds of deoxy nucleotides [dATP, dGTP, dCTP, dTTP, and 15 mM(s) each] -- each 5 mul It added every. Subsequently, primer solution 5 of 20 bases which face across a field to carry out gene amplification, and have the base sequence of the antisense strand of 5 '5micro [of primer solution of 20 bases with the base sequence of the sense chain of the upstream] (100ng/mul) I and further 3' downstream mul (100ng/mul) is added and it is water 0.7 to the last. mul It is whole-quantity 49microl moreover. It considered as the solution. This solution is processed for 5 minutes at 92 degrees

C, and it cools to a room temperature, and is Taq. Polymerase 1 mul (two units and New England Biolabs shrine product) was added. The following, annealing (55 **, 45 seconds), polymerization (72 **, 2 minutes), and denaturation (90 **, 1 minute) DNA was amplified repeatedly 35 times.

[0034] H7 of the gene product amplified by RT-PCR method About a fragment, it is agarose gel (2%). Electrophoresis was carried out and DNA of the target die length was collected. Subsequently, it is Klenow fragment about this. Enzyme processing is carried out, the end of DNA is arranged flat and smooth, and it is T four further. The five prime end was phosphorized by the polynucleotide kinase. This was inserted in the HincII part of plasmid vector pTZ19R, and the gene was cloned. In this way, it rearranges and is a plasmid pHCV7. It obtained.

[0035] Recombination plasmid pHCV7 It is displayed as E.coli HCV7 and the Escherichia coli by which the transformation was carried out is the Fermentation Research Institute mycoparasite 11831st to the Fermentation Research Institute, the Agency of Industrial Science and Technology, the Ministry of International Trade and Industry. It ****s as a number.

(2-2) Escherichia coli HB one 101 Production pHCV7 of [pCI07] EcoRI It digests by StuI and they are 338bp(s) by the side of 5' of cDNA. The fragment was obtained. These 338bp(s) A fragment is HinfI further. An end is graduated by DNA polymerase I Klenow fragment after partial digestion, and it is 263bp. The fragment was obtained. Moreover, pHCV7 After digesting and carrying out CIP processing by StuI, PstI digestion is carried out, and they are 400bp(s) by the side of 3' of cDNA. The fragment was obtained. On the other hand, it is pUEX1 (product made from Amersham). It digests by SmaI and PstI and is CIP. It processed. This pUEX1 400bp(s) by the side of 5 'the near 263bp fragment and 3 of cDNA' of cDNA The ligation reaction of a fragment is performed and it is the recombination vector pCI07. It obtained. an example 1 -- the same -- carrying out -- a base sequence -- determining -- this recombination vector pCI07 **** -- it counts from the amino terminal of HCV, the base sequence which carries out the code of the 1323rd to the 1533rd amino acid sequence is included, and that base sequence is as being shown in the array number 6. Next, recombination vector pCI07 Host Escherichia coli HB one 101 A transformation is carried out and it is recombination Escherichia coli HB one 101. [pCI07] was obtained. Recombination Escherichia coli HB one 101 [pCI07] was cultivated at 30 degrees C by the LB+Amp culture medium overnight, the glycerol was added and cryopreservation was carried out at -80 degrees C so that the last concentration might become 15%.

(2-3) Manufacture recombination Escherichia coli HB one 101 of NS-3 antigen By cultivating [pCI07] and performing gene expression, NS-3 antigen is produced as a fusion polypeptide with the beta-galactosidase. It is recombination Escherichia coli HB one 101 like manufacture of the Core (1-3) antigen of an example 1. Culture of [pCI07], crushing of a fungus body, and separation purification of a fusion polypeptide were performed. It cultivates in a 20l. LB+Amp culture medium, and is 1,000mg. NS-3 antigen was obtained. It checked that it was in agreement with the molecular weight (141kd) which investigates molecular weight by SDS-PAGE and is calculated from the amino acid sequence about NS-3 obtained antigen.

[0036] (Example 3) The cDNA library obtained in the production example 1 of the manufacture (3-1) recombination plasmid pHCV10 of NS-4 antigen was screened by plaque hybridization. first -- Escherichia coli Y1090 -- a host -- carrying out -- ten plates with a diameter of 15cm -- recombination lambdagt11 phage 5x105 of a cDNA library an equivalent -- it was made to appear The obtained plaque was copied to the nitrocellulose and hybridization was performed. In this way, six shares of clones with a HCV gene fragment were chosen. And phage DNA is collected from this clone and, subsequently it is EcoRI. It cut and six kinds of HCV gene fragments, H1, H5, H10, H13 and H20, and H21 fragments were collected from agarose electrophoresis gel. Among these, it is EcoRI of plasmid vector pTZ19R about this fragment about H10 fragment including the base sequence which carries out the code of the indispensable amino acid sequence to the outstanding antigen activity. It inserted in the part and the recombination plasmid pHCV10 was obtained.

[0037] The Escherichia coli in which the transformation was carried out by the recombination plasmid pHCV10 is E.coli HCV10. It displays and is the Fermentation Research Institute mycoparasite 11834th to the Fermentation Research Institute, the Agency of Industrial Science and Technology, the Ministry of International Trade and Industry. It ****s as a number.

(3-2) Escherichia coli HB one 101 It is AvaII about the production pHCV10 of [pCI10]. An end is graduated by DNA polymerase I Klenow fragment after digestion, and it is BamHI further. It digests and they are 583bp(s). The fragment was isolated. On the other hand, it is pUEX3 (product made from Amersham). It digests by SmaI and is CIP. It processes and is BamHI further. It digested. Then, electrophoresis was performed and the target fragment was separated. Ligation of these is carried out and it is the recombination vector pCI10. It produced. an example 1 -- the same -- carrying out -- a base sequence -- determining -- this recombination vector pCI10 **** -- it counts from the amino terminal of HCV and the base sequence which carries out the code of the 1605th to the 1798th amino acid sequence is included, and that base sequence comes out as it is shown in the array number 7.

Next, recombination vector pCI10 Host Escherichia coli HB one 101 A transformation is carried out and it is recombination Escherichia coli HB one 101. [pCI10] was obtained. Recombination Escherichia coli HB one 101 [pCI10] was cultivated at 30 degrees C by the LB+Amp culture medium overnight, the glycerol was added and cryopreservation was carried out at -80 degrees C so that the last concentration might become 15%.
(3-3) Manufacture recombination Escherichia coli HB one 101 of NS-4 antigen By cultivating [pCI10] and performing gene expression, NS-4 antigen is produced as a fusion polypeptide with the beta-galactosidase. It is recombination Escherichia coli HB one 101 like manufacture of the Core (1-3) antigen of an example 1. Culture of [pCI10], crushing of a fungus body, and separation purification of a fusion polypeptide were performed. It cultivates in a 20l. LB+Amp culture medium, and is 720mg. NS-4 antigen was obtained. It checked that it was in agreement with the molecular weight (141kd) which investigates molecular weight by SDS-PAGE and is calculated from the amino acid sequence about NS-4 obtained antigen.
[0038] (Example 4) It is agarose gel (2%) about H14 fragment of the gene product of NS5 field of HCV amplified by RT-PCR method like the production example 2 of the manufacture (4-1) recombination plasmid pHCV14 of NS-5 antigen. Electrophoresis was carried out and DNA of the target die length was collected. In addition, the base sequence of CGGGCATGACCACTGACAA(5') C (3') and 3' downstream primer of the base sequence of 5' upstream primer is CCGCCTCTAGGACGCTTTT(5') G (3'). Subsequently, it is Klenow fragment about this. Enzyme processing is carried out, the end of DNA is arranged flat and smooth, and it is T four further. The five prime end was phosphorized by the polynucleotide kinase. This was inserted in the HincII part of plasmid vector pTZ19R, and the recombination plasmid pHCV14 was obtained. The Escherichia coli in which the transformation was carried out by the recombination plasmid pHCV14 is E.coli HCV14. It displays and is the Fermentation Research Institute mycoparasite 11838th to the Fermentation Research Institute, the Agency of Industrial Science and Technology, the Ministry of International Trade and Industry. It ****s as a number.
(4-2) Escherichia coli HB one 101 The end was graduated for the production pHCV14 of [pCI14] by blunting kit after digestion by PstI and XbaI, and the fragment containing 484bp was isolated. On the other hand, it is pUEX2. It digests by SmaI and is CIP. It processed. Then, electrophoresis was performed and the target fragment was separated. Ligation of these is carried out and it is the recombination vector pCI14. It produced. an example 1 — the same — carrying out — a base sequence — determining — this recombination vector pCI14 **** — it counts from the amino terminal of HCV, the base sequence which carries out the code of the 2111st to the 2270th amino acid sequence is included, and that base sequence is as being shown in the array number 8. Next, recombination vector pCI14 Host Escherichia coli HB one 101 A transformation is carried out and it is recombination Escherichia coli HB one 101. [pCI14] was obtained. Recombination Escherichia coli HB one 101 [pCI14] was cultivated at 30 degrees C by the LB+Amp culture medium overnight, the glycerol was added and cryopreservation was carried out at -80 degrees C so that the last concentration might become 15%.
(4-3) Manufacture recombination Escherichia coli HB one 101 of NS-5 antigen By cultivating [pCI14] and performing gene expression, NS-5 antigen is produced as a fusion polypeptide with the beta-galactosidase. It is recombination Escherichia coli HB one 101 like manufacture of the Core (1-3) antigen of an example 1. Culture of [pCI14], crushing of a fungus body, and separation purification of a fusion polypeptide were performed. It cultivates in a 20l. LB+Amp culture medium, and is 750mg. NS-5 antigen was obtained. It checked that it was in agreement with the molecular weight (135kd) which investigates molecular weight by SDS-PAGE and is calculated from the amino acid sequence about NS-4 antigen obtained about NS-5 obtained antigen.
[0039] (Example 5) Immunological agglutination reaction reagent for a hepatitis C diagnosis using a HCV antigen activity polypeptide (heating actuation) Respectively, carry out equivalent distribution in 100micro everyg/ml at PBS, and let four sorts of HCV antigen activity polypeptides, a Core antigen, NS-3 antigen, NS-4 antigen, and NS-5 antigen, be mixed antigen solutions. HDP (Tokuyama Soda Co., Ltd. product) with a diameter of 1.8 micrometers which heats this antigen mixed solution for 30 minutes at 35 degrees C, and is used as the antigen solution for sensitization (sensitization) was suspended so that it might become 5(weight/weight) % by PBS, and it considered as HDP suspension. The 1ml of the above-mentioned HDP suspension and 1ml of antigen solutions for sensitization which performed heating actuation were mixed within the test tube, it was left at the room temperature for 1 hour, and four kinds of HCV antigen activity polypeptides were made to stick to a HDP front face in canal (this adsorption actuation is hereafter written also as sensitization).
[0040] (Washing actuation) In order to remove an excessive HCV antigen activity polypeptide after that, at-long-intervals alignment separation was given to the above-mentioned mixed liquor for 2,500rpm and 5 minutes, and centrifugal supernatant liquid was removed. For washing to the centrifugation precipitate, addition, after suspension] 2,500rpm, and after [a 5 minute alignment at long intervals] supernatant liquid were removed, and PBS2ml was suspended so that it might become 0.5(w/vol) % to 3(vol/vol) % denaturation rabbit blood serum content PBS (it is hereafter written also as A liquid). HDP (it is hereafter written also as a sensitization particle) to which the above and a HCV polypeptide were made to stick was used as the immunological agglutination

reaction reagent for a hepatitis C diagnosis (it is hereafter written also as B liquid).
[0041] (Measurement actuation) On the other hand, from twice, two-fold dilution of the specimen used for inspection was carried out, and it was diluted with A liquid up to 8192 times. Next, the diluent of a specimen was respectively dropped at 96 hole microtiter plate (96 well micro-titer-plate) from one hole to 12 holes every [25micro / l]. Subsequently, 25micro of each hole l was dropped for B liquid prepared above. After dropping, the sedimentation pattern was observed, after shaking by the plate mixer (plate mixer) and putting for 30 minutes. Since the antigen-antibody reaction arose among sedimentation patterns, that to which the sensitization particle spread in the tube bottom of a microplate was made into the hot nodule, and since an antigen-antibody reaction did not arise, it made for the sensitization particle to have precipitated to the tube bottom of a microtiter plate into the cold nodule. Generally, since detection sensitivity is displayed for the highest dilution scale factor (it is hereafter written also as a potency) of the blood serum with which the hot nodule is observed, and plasma, the sensibility of the immunological agglutination reaction reagent for a hepatitis C diagnosis is hereafter expressed as a microtiter reagent with a potency. In addition, by the healthy person specimen, this potency is low and is made better, as high by the patient specimen.
[0042] (Result) The commercial item A which is next the ELISA method reagent was compared with the immunological agglutination reaction reagent for a hepatitis C diagnosis prepared in the example 5. Healthy person specimen 5 specimen and patient's-serum 5 specimen were used for examination. Although the commercial item A and the immunological agglutination reaction reagent for a hepatitis C diagnosis of this invention showed good correlation, as for one specimen, only the immunological agglutination reaction reagent for this hepatitis C diagnosis showed the positivity. In addition, a positivity and negative decision made the positivity what shows the hot nodule from dilution 32 times (refer to Table 1).
[0043] Further 30 specimens were measured for the healthy person specimen and the patient specimen (refer to Table 2). Two specimens of specimens out of which a negative judgment with a positivity and a commercial item A comes with the immunological agglutination reaction reagent for a hepatitis C diagnosis of an example 5 were detected.
[0044]
[Table 1]

実施例５と市販品Ａの比較（１）

検体 No.	感度	
	実施例５	市販品Ａ (OD492)
1	>8192	++(>2.00)
2	8192	++(>2.00)
3	512	++(>2.00)
4	2048	++(>2.00)
5	64	-(0.114)
6	8	-(0.017)
7	16	-(0.042)
8	8	-(0.023)
9	16	-(0.006)
10	8	-(0.003)

[0045]
[Table 2]

実施例５と市販品Ａの比較（２）

		実施例５	
		健常者検体	患者検体
市販品Ａ	陰性	14	2
	陽性	0	14

[0046] Example 1 of a comparison Respectively, equivalent distribution is carried out in 100micro everyg/ml at PBS, the mixed antigen solution of four sorts of HCV antigen activity polypeptides, the immunological agglutination reaction reagent (mixed actuation) Core antigen for a hepatitis C diagnosis using the HCV antigen activity polypeptide which does not heat-treat, NS-3 antigen, NS-4 antigen, and NS-5 antigen, is carried out, and it considers as the antigen solution for sensitization.
[0047] (Sensitization) HDP (Tokuyama Soda Co., Ltd. product) with a diameter of 1.8 micrometers was suspended so that it might become 5(w/w) % by PBS, and it considered as HDP suspension. The 1ml of the above-mentioned antigen liquid for sensitization was put in in the test tube, and sensitization of the HCV antigen activity polypeptide which mixed with HDP suspension, left at the room temperature for 1 hour, and was mixed on the HDP front face was carried out.
[0048] (Washing actuation) The excessive HCV antigen activity polypeptide was removed by the same actuation as an example 5, and it suspended so that it might become particle concentration 0.5(w/vol) % with A liquid. The above and a sensitization particle were used as the immunological agglutination reaction reagent for a hepatitis C diagnosis (it is hereafter written also as C fluid).
(Measurement actuation) Two-fold dilution of the specimen used for inspection like an example 5 was carried out from twice with A liquid, it diluted up to 8192 times, and the sedimentation pattern was observed after dropping C fluid, D liquid, E liquid, and F liquid.
[0049] (Result) The specimen (it is hereafter written also as the healthy person specimen 1) they are the specimen (it is hereafter written also as the patient specimen 1) it is 8192 times whose potency of this, and 8 times whose potency of this was used for examination with the immunological agglutination reaction reagent for a hepatitis C diagnosis of an example 5. The potency lower than the immunological agglutination reaction reagent for a hepatitis C diagnosis which the immunological agglutination reaction reagent for a hepatitis C diagnosis which carried out sensitization of the above-mentioned HCV antigen activity polypeptide prepared in the example 5 was shown (refer to Table 3).
[0050]
[Table 3]

実施例5と比較例1の比較

	感　度	
	健常者検体1	患者検体1
実施例5	x8	x8192
比較例1	x8	x2048

[0051] Example 2 of a comparison The immunological agglutination reaction reagent (heat-treatment) Core antigen for a hepatitis C diagnosis using one kind of HCV antigen activity polypeptide, Four sorts of HCV antigen activity polypeptides of NS-3 antigen, NS-4 antigen, and NS-5 antigen are distributed to PBS so that it may become in ml and 100microg /respectively. It heat-treated for 30 minutes at 35 degrees C, and the Core antigen liquid for sensitization, the NS-3 antigen liquid for sensitization, the NS-4 antigen liquid for sensitization, and the NS-5 antigen liquid for sensitization were prepared, respectively.
[0052] (Sensitization) HDP (Tokuyama Soda Co., Ltd. product) with a diameter of 1.8 micrometers was suspended so that it might become 5(w/w) % by PBS, and it considered as HDP suspension. The above-mentioned Core antigen liquid for sensitization, the NS-3 antigen liquid for sensitization, the NS-4 antigen liquid for sensitization, and 1ml of each NS-5 antigen liquid for sensitization were put in in the separate test tube, and it mixed with HDP suspension, was left at the room temperature for 1 hour, and sensitization of each HCV antigen activity polypeptide was carried out to the HDP front face.
[0053] (Washing actuation) The excessive HCV antigen activity polypeptide was removed by the same actuation as an example 5, and it suspended so that it might become particle concentration 0.5(w/vol) % with A liquid. The above and a sensitization particle were used as the immunological agglutination reaction reagent for a hepatitis C diagnosis (it is hereafter written also as D, E, F, and G liquid).
(Measurement actuation) Two-fold dilution of the specimen used for inspection like an example 5 was carried out from twice with A liquid, it diluted up to 8192 times, and the sedimentation pattern was observed after dropping D liquid, E liquid, F liquid, and G liquid.
[0054] (Result) The patient specimen 1 and the healthy person specimen 1 were used for examination. The potency lower than the immunological agglutination reaction reagent for a hepatitis C diagnosis which the

immunological agglutination reaction reagent for a hepatitis C diagnosis which carried out sensitization of the above-mentioned HCV antigen activity polypeptide prepared in the example 5 was shown (refer to Table 4).

[0055]

[Table 4]

実施例5と比較例2の比較

抗原		感度	
		健常者検体1	患者検体1
実施例5	GCC-Core, NS-3 NS-4, NS-5	x8	x8192
比較例2	Core	x8	x256
	NS-3	x8	x128
	NS-4	x8	x64
	NS-5	x8	x128

[0056] Example 3 of a comparison The immunological agglutination reaction reagent (heat-treatment) Core antigen for a hepatitis C diagnosis and NS-3 antigen using two kinds of HCV antigen activity polypeptides, A Core antigen, NS-4 antigen and a Core antigen, NS-5 antigen and NS-3 antigen, and NS-4 antigen, Equivalent distribution of every two kinds of HCV antigen activity polypeptides of NS-3 antigen, NS-5 antigen and NS-4 antigen, and NS-5 antigen is respectively carried out in 50micro everyg/ml at PBS, and for 35 degrees C and 30 minutes, heat-treatment is carried out and it considers as the antigen solutions 1, 2, 3, 4, 5, and 6 for sensitization, respectively.

[0057] (Sensitization) HDP (Tokuyama Soda Co., Ltd. product) with a diameter of 1.8 micrometers was suspended so that it might become 5(w/w) % by PBS, and it considered as HDP suspension. The 1ml of the above-mentioned HDP suspension and 1ml of antigen solutions for sensitization were mixed within the test tube, it was left at the room temperature for 1 hour, and sensitization of two kinds of HCV antigen activity polypeptides was respectively carried out to the HDP front face.

[0058] (Washing actuation) The excessive HCV antigen activity polypeptide was removed by the same actuation as an example 5, and it suspended so that it might become particle concentration 0.5(w/vol) % with A liquid. The above and a sensitization particle were used as the immunological agglutination reaction reagent for a hepatitis C diagnosis (it is hereafter written also as H, I, J, K, L, and M liquid).

[0059] (Measurement actuation) Two-fold dilution of the patient specimen 1 and the healthy person specimen 1 which are used for inspection like an example 5 was carried out from twice with A liquid, it diluted up to 8192 times, and the sedimentation pattern was observed after dropping H, I, J, K, L, and M liquid.

(Result) The potency lower than the immunological agglutination reaction reagent for a hepatitis C diagnosis which the immunological agglutination reaction reagent for a hepatitis C diagnosis which carried out sensitization of two kinds of HCV antigen activity polypeptides prepared in the example 5 was shown respectively (refer to Table 5).

[0060]

[Table 5]

実施例5比較例3の比較

抗原		感度	
		健常者検体1	患者検体1
実施例5	GCC-Core, NS-3 NS-4, NS-5	x8	x8192
比較例3	Core, NS-3	x8	x256
	Core, NS-4	x8	x128
	Core, NS-5	x8	x64
	NS-3, NS-4	x8	x64
	NS-3, NS-5	x8	x128
	NS-4, NS-5	x8	x64

[0061] Example 4 of a comparison The immunological agglutination reaction reagent (heat-treatment) Core antigen for a hepatitis C diagnosis and NS-3 antigen using three kinds of HCV antigen activity polypeptides, and NS-4 antigen, Equivalent distribution of every three kinds of HCV antigen activity polypeptides of a Core antigen, NS-3 antigen, NS-5 antigen and a Core antigen, NS-4 antigen, NS-5 antigen and NS-3 antigen, NS-4 antigen, and NS-5 antigen is respectively carried out in 33micro everyg/ml at PBS. 35 degrees C, Heat-treatment for 30 minutes is performed and it considers as the antigen solutions 7, 8, 9, and 10 for sensitization.
[0062] (Sensitization) HDP (Tokuyama Soda Co., Ltd. product) with a diameter of 1.8 micrometers was suspended so that it might become 5(w/w) % by PBS, and it considered as HDP suspension. The 1ml of the above-mentioned HDP suspension and 1ml of each antigen solutions for sensitization were mixed within the test tube, it was left at the room temperature for 1 hour, and sensitization of three kinds of HCV antigen activity polypeptides was respectively carried out to the HDP front face.
[0063] (Washing actuation) The excessive HCV antigen activity polypeptide was removed by the same actuation as an example 5, and it suspended so that it might become particle concentration 0.5(w/vol) % with A liquid. The above and a sensitization particle were used as the immunological agglutination reaction reagent for a hepatitis C diagnosis (it is hereafter written also as N, O, P, and Q liquid).
(Measurement actuation) Two-fold dilution of the patient specimen 1 and the healthy person specimen 1 which are used for inspection like an example 5 was carried out from twice with A liquid, it diluted up to 8192 times, and the sedimentation pattern was observed after dropping N0, P, and Q liquid.
[0064] (Result) The potency lower than the immunological agglutination reaction reagent for a hepatitis C diagnosis which the immunological agglutination reaction reagent for a hepatitis C diagnosis which carried out sensitization of three kinds of HCV antigen activity polypeptides prepared in the example 5 was shown respectively (refer to Table 6).
[0065]
[Table 6]

実施例5と比較例4の比較

抗原		感度	
		健常者検体1	患者検体1
実施例5	GCC-Core, NS-3 NS-4, NS-5	x8	x8192
比較例4	Core, NS-3, NS-4	x8	x1024
	Core, NS-3, NS-5	x8	x512
	Core, NS-4, NS-5	x8	x128
	NS-3, NS-4, NS-5	x8	x64

[0066] (Example 6) 100ml of immunological agglutination reaction reagent (immobilization of sheep erythrocyte) sheep erythrocytes for a hepatitis C diagnosis and 100ml of ORUSEBA liquid using sheep erythrocyte support were mixed, and the physiological saline washed after corpuscle density measurement after gauze filtration. Formalin fixation was performed about the above-mentioned corpuscle (based on Williams, C, and Academic Press New York edited by Hase). (Methods in Immunology and Immunochemistory, vol, and pp 33-34 (1977)) (Heat-treatment) Heat-treatment was performed for the solution which carried out equivalent mixing of four sorts of HCV antigen activity polypeptides, a Core antigen, NS-3 antigen, NS-4 antigen, and NS-5 antigen, ml 100micro everyg /respectively by PBS like the example 5 for 35 degrees C and 30 minutes (it is hereafter written also as R liquid).
[0067] (Sensitization) It is 3(vol/vol) % formalin to a washed erythrocyte. It is 40(vol/vol) % formalin to the pan after adding physiological saline liquid and stirring at 10 degrees C for 24 hours. Physiological saline liquid was added and it stirred for 24 hours. After the physiological saline washed, it suspended so that it might become 2.5 (vol/vol) %, and considered as the fixed sheep erythrocyte.
[0068] It was made to react for 1 hour, stirring the 1ml of the above-mentioned Q liquid, and 1ml of fixed sheep erythrocyte solutions diluted with PBS to 5(w/w) % at 37 degrees C. Sensitization of four sorts of HCV antigen activity polypeptides was carried out to the fixed sheep erythrocyte by this actuation.
(Washing actuation) Washing actuation was performed as it is subsequently the same as that of an example 5, the excessive HCV antigen activity polypeptide was removed, and it suspended so that it might become particle concentration 0.5(w/vol) % at A liquid. The above and a sensitization particle were used as the immunological agglutination reaction reagent for a hepatitis C diagnosis (it is hereafter written also as S liquid).

[0069] (Measurement actuation) Two-fold dilution of the patient specimen 1 and the healthy person specimen 1 which are used for inspection like an example 5 was carried out from twice with A liquid, it diluted up to 8192 times, and the sedimentation pattern was observed after dropping R liquid.
(Result) The commercial item A was compared with the immunological agglutination reaction reagent for a hepatitis C diagnosis prepared in the example 6 like the example 5. Healthy person specimen 5 specimen and patient's-serum 5 specimen were used for examination. Although the commercial item A and the immunological agglutination reaction reagent for this hepatitis C diagnosis showed good correlation, as for one specimen, only the immunological agglutination reaction reagent for this hepatitis C diagnosis showed the positivity. In addition, a positivity and negative decision made the positivity what shows the hot nodule from dilution 32 times (refer to Table 7).
[0070]
[Table 7]

実施例 6 と市販品Aの比較

検体 No.	感度	
	実施例 6	市販品A (OD492)
1	>8192	++(>2.00)
2	8192	++(>2.00)
3	256	++(>2.00)
4	2048	++(>2.00)
5	128	-(0.136)
6	16	-(0.027)
7	16	-(0.046)
8	8	-(0.043)
9	8	-(0.016)
10	8	-(0.003)

[0071] Example 5 of a comparison After distributing the comparison (heat-treatment) Core antigen of an ELISA reagent and an immunological agglutination reaction reagent, NS-3 antigen, NS-4 antigen, and NS-5 antigen by PBS so that it may become [ml] in 100microg /, 35 degrees C and heat-treatment for 30 minutes were performed. Let each be a heat-treatment Core antigen, heat-treatment NS-3 antigen, heat-treatment NS-4 antigen, and heat-treatment NS-5 antigen. Moreover, after carrying out equivalent mixing of a Core antigen, NS-3 antigen, NS-4 antigen, and the NS-5 antigen ml 25micro everyg /, 35 degrees C and heat-treatment for 30 minutes are performed, and it considers as a heat-treatment antigen solution.
[0072] (Adsorption on a microtiter plate) The heat-treatment Core antigen, heat-treatment NS-3 antigen, heat-treatment NS-4 antigen, and heat-treatment NS-5 antigen were poured distributively every [50micro / per hole / l] on the microtiter plate (Nunc product). This heat-treatment antigen solution was similarly poured distributively every [50micro / per hole / l] on the microtiter plate. The above-mentioned microtiter plate was made to adsorb at 37 degrees C for 1 hour. It washed 3 times by PBS and 200microl after adsorption.
[0073] (Blocking actuation) 50micro (it is hereafter written also as a BSA solution) of bovine blood albumin content PBS was poured distributively l times 1% on this microtiter plate in each hole, and it blocked at 37 degrees C for 1 hour. The BSA solution was removed after blocking.
(Primary antibody reactions) after diluting 10micro of patient specimen 1 and healthy person specimen 1 each l with a BSA solution 10 times, it poured distributively in each hole and was made to react at 37 degrees C for 1 hour
[0074] (Washing actuation) The diluent of each specimen was removed after primary antibody reaction termination. 200micro (it is hereafter written also as a penetrant remover) of Tween 80 (tween80) content PBS solutions l washed 3 times 0.5%.
(Secondary antibody reactions) peroxidase labelling anti-Homo sapiens IgG (Cappel product) who diluted with the BSA solution 20,000 times was poured distributively in 100microl [every] each hole, and it was made to react at 37 degrees C for 1 hour
[0075] (Washing actuation) The diluent of each specimen was removed after primary antibody reaction termination. 200micro (it is hereafter written also as a penetrant remover) of Tween 80 (Tween80) content PBS solutions l washed 3 times 0.5%.
(Coloring actuation) The equivalent mixed liquor of a hydrogen-peroxide-solution solution (Cappel product) and an ABTS solution (Cappel product) was made to react for 30 minutes at a room temperature after adding 100microl. After the reaction, 100microl was added for the sodium-dodecyl-sulfate solution 10%, the reaction

was suspended, and the absorbance (wavelength of 414nm) of this reaction mixture was measured.
[0076] (Result) Although the hole to which the Core antigen, NS-3 antigen, NS-4 antigen, and NS-5 antigen were made to stick, respectively was compared with the hole which mixed these four kinds of HCV antigen activity polypeptides, the reagent for a hepatitis C diagnosis of high sensitivity as which the absorbance was equivalent to and was regarded with the agglutination reaction reagent always was not able to be prepared (refer to Table 8).
[0077]
[Table 8]

比較例5の結果

抗原	感度	
	健常者検体1 (OD414)	患者検体1 (OD414)
GCC-Core, NS-3 NS-4, NS-5	0.134	1.999
Core	0.111	1.922
NS-3	0.169	1.694
NS-4	0.158	1.825
NS-5	0.185	1.770

[0078]
[Layout Table]
1. Die-Length [of Array Number 1 (1) Array]: — Mold [of 168 (2) Arrays]: — Amino Acid (3) Topology: — Class [of Straight Chain-like (4) Array]: — Protein (5) Origin Living Thing Name: — HCV (Hepatitis C Virus)

(6) 配列

```
Met Ser Thr Asn Pro Lys Pro Gln Arg Lys
                5                  10
Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln
               15                  20
Asp Val Lys Phe Pro Gly Gly Gly Gln Ile
               25                  30
Val Gly Gly Val Tyr Leu Leu Pro Arg Arg
               35                  40
Gly Pro Arg Leu Gly Val Arg Ala Thr Arg
               45                  50
Lys Thr Ser Glu Arg Ser Gln Pro Arg Gly
               55                  60
Arg Arg Gln Pro Ile Pro Lys Asp Arg Arg
               65                  70
Ser Thr Gly Lys Ser Trp Gly Lys Pro Gly
               75                  80
Tyr Pro Trp Pro Leu Tyr Gly Asn Glu Gly
               85                  90
Cys Gly Trp Ala Gly Trp Leu Leu Ser Pro
               95                 100
Arg Gly Ser Arg Pro Thr Trp Gly Pro Thr
              105                 110
Asp Pro Arg His Arg Ser Arg Asn Leu Gly
              115                 120
Lys Val Ile Asp Thr Ile Thr Cys Gly Phe
              125                 130
Ala Asp Leu Met Gly Tyr Ile Pro Val Val
              135                 140
Gly Ala Pro Val Gly Gly Val Ala Arg Ala
              145                 150
Leu Ala His Gly Val Arg Val Leu Glu Asp
              155                 160
Gly Val Asn Tyr Ala Thr Gly Asn
              165
```

2. Die-Length [of Array Number 2 (1) Array]: — Mold [of 211 (2) Arrays]: — Amino Acid (3) Topology: — Class [of Straight Chain-like (4) Array]: — Protein (5) Origin Living Thing Name: — HCV (Hepatitis C Virus)

(6) 配列

Ser Thr Thr Ile Leu Gly Ile Gly Thr Val
5 10
Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg
15 20
Leu Val Val Leu Ala Thr Ala Thr Pro Pro
25 30
Gly Ser Ile Thr Val Pro His Pro Asn Ile
35 40
Glu Glu Val Ala Leu Ser Asn Thr Gly Glu
45 50
Ile Pro Phe Tyr Gly Lys Ala Ile Pro Ile
55 60
Glu Ala Ile Lys Gly Gly Arg His Leu Ile
65 70
Phe Cys His Ser Lys Lys Lys Cys Asp Glu
75 80
Leu Ala Ala Lys Leu Thr Gly Leu Gly Leu
85 90
Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp
95 100
Val Ser Val Ile Pro Thr Ser Gly Asp Val
105 110
Val Val Val Ala Thr Asp Ala Leu Met Thr
115 120
Gly Phe Thr Gly Asp Phe Asp Ser Val Ile
125 130
Asp Cys Asn Thr Cys Val Thr Gln Thr Val
135 140
Asp Cys Ser Leu Asp Pro Thr Phe Thr Ile
145 150
Glu Thr Thr Thr Val Pro Gln Asp Ala Val
155 160
Ser Arg Pro Gln Arg Arg Gly Arg Thr Gly
165 170
Arg Gly Arg Ser Gly Ile Tyr Arg Phe Val
175 180
Thr Pro Gly Glu Arg Pro Ser Gly Met Phe
185 190
Asp Ser Ser Val Leu Cys Glu Cys Tyr Asp
195 200
Ala Gly Cys Ala Trp Tyr Glu Leu Thr Pro
205 210
Ala

3. Die-Length [of Array Number 3 (1) Array]: -- Mold [of 194 (2) Arrays]: -- Amino Acid (3) Topology: -- Class [of Straight Chain-like (4) Array]: -- Protein (5) Origin Living Thing Name: -- HCV (Hepatitis C Virus)

(6) 配列

```
Asp Gln Met Trp Lys Cys Leu Ile Arg Leu
                  5                  10
Lys Pro Thr Leu His Gly Pro Thr Pro Leu
                 15                  20
Leu Tyr Arg Leu Gly Ala Val Gln Asn Glu
                 25                  30
Val Thr Leu Thr His Pro Ile Thr Lys Tyr
                 35                  40
Ile Met Ala Cys Met Ser Ala Asp Leu Glu
                 45                  50
Val Val Thr Ser Thr Trp Val Leu Val Gly
                 55                  60
Gly Val Leu Ala Ala Leu Ala Ala Tyr Cys
                 65                  70
Leu Thr Thr Gly Ser Val Val Ile Val Gly
                 75                  80
Arg Ile Ile Leu Ser Gly Arg Pro Ala Val
                 85                  90
Ile Pro Asp Arg Glu Val Leu Tyr Gln Glu
                 95                 100
Phe Asp Glu Met Glu Glu Cys Ala Ser His
                105                 110
Leu Pro Tyr Ile Glu Gln Gly Met Gln Leu
                115                 120
Ala Glu Gln Phe Lys Gln Lys Ala Leu Gly
                125                 130
Leu Leu Gln Thr Ala Thr Lys Gln Ala Glu
                135                 140
Ala Ala Ala Pro Val Val Glu Ser Lys Trp
                145                 150
Arg Ala Leu Glu Val Phe Trp Ala Lys His
                155                 160
Met Trp Asn Phe Ile Ser Gly Ile Gln Tyr
                165                 170
Leu Ala Gly Leu Ser Thr Leu Pro Gly Asn
                175                 180
Pro Ala Ile Ala Ser Leu Met Ala Phe Thr
                185                 190
Ala Ser Ile Thr
```

4. Die-Length [of Array Number 4 (1) Array]: — Mold [of 160 (2) Arrays]: -- Amino Acid (3) Topology: -- Class [of Straight Chain-like (4) Array]: — Protein (5) Origin Living Thing Name: — HCV (Hepatitis C Virus)

(6) 配列

```
Lys Cys Pro Cys Gln Val Pro Ala Pro Glu
                5                  10
Phe Phe Thr Glu Val Asp Gly Val Arg Leu
               15                  20
His Arg Tyr Ala Pro Val Cys Lys Pro Leu
               25                  30
Leu Arg Glu Glu Val Val Phe Gln Val Gly
               35                  40
Leu Asn Gln Tyr Leu Val Gly Ser Gln Leu
               45                  50
Pro Cys Glu Pro Glu Pro Asp Val Ala Val
               55                  60
Leu Thr Ser Met Leu Thr Asp Pro Ser His
               65                  70
Ile Thr Ala Glu Met Ala Lys Arg Arg Leu
               75                  80
Ala Arg Gly Ser Pro Pro Ser Leu Ala Ser
               85                  90
Ser Ser Ala Ser Gln Leu Ser Ala Pro Ser
               95                 100
Leu Lys Ala Thr Cys Thr Thr His His Asp
              105                 110
Ser Pro Asp Ala Asp Leu Ile Glu Ala Asn
              115                 120
Leu Leu Trp Arg Gln Glu Met Gly Gly Asn
              125                 130
Ile Thr Arg Val Glu Ser Glu Asn Lys Val
              135                 140
Val Ile Leu Asp Ser Phe Asp Pro Ile Arg
              145                 150
Ala Val Glu Asp Glu Arg Glu Val Ser Val
              155                 160
```

5. Die-Length [of Array Number 5 (1) Array]: — Mold [of 504 (2) Arrays]: — Number [of Nucleic-Acid (3) Chains]: — Double Strand (4) Topology: — Class [of Straight Chain-like (5) Array]: — CDNA to Genomic RNA (6) Origin Living Thing Name: — HCV (Hepatitis C Virus)
(7) The notation:peptide existence location showing the description:description which is an array : approach:E

(8) 配列

```
ATG AGC ACA AAT CCT AAA CCT CAA AGA AAA  30
Met Ser Thr Asn Pro Lys Pro Gln Arg Lys
                5                  10
ACC AAA CGT AAC ACC AAC CGC CGC CCA CAG  60
Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln
               15                  20
GAC GTT AAG TTC CCG GGT GGC GGT CAG ATC  90
Asp Val Lys Phe Pro Gly Gly Gly Gln Ile
               25                  30
GTT GGC GGA GTT TAC CTG CTG CCG CGC AGG 120
Val Gly Gly Val Tyr Leu Leu Pro Arg Arg
               35                  40
GGC CCC AGG TTG GGT GTG CGC GCG ACA AGG 150
Gly Pro Arg Leu Gly Val Arg Ala Thr Arg
               45                  50
AAG ACT TCC GAG CGA TCC CAG CCG CGT GGA 180
Lys Thr Ser Glu Arg Ser Gln Pro Arg Gly
               55                  60
AGA CGC CAG CCC ATC CCG AAA GAT AGG CGC 210
Arg Arg Gln Pro Ile Pro Lys Asp Arg Arg
               65                  70
TCC ACC GGC AAG TCC TGG GGA AAG CCA GGA 240
Ser Thr Gly Lys Ser Trp Gly Lys Pro Gly
               75                  80
TAT CCT TGG CCT CTG TAT GGA AAC GAG GGT 270
Tyr Pro Trp Pro Leu Tyr Gly Asn Glu Gly
               85                  90
TGC GGC TGG GCA GGT TGG CTC CTG TCC CCC 300
Cys Gly Trp Ala Gly Trp Leu Leu Ser Pro
               95                 100
CGC GGA TCT CGT CCT ACT TGG GGC CCC ACT 330
Arg Gly Ser Arg Pro Thr Trp Gly Pro Thr
              105                 110
GAC CCC CGG CAC AGA TCG CGC AAT TTG GGC 360
Asp Pro Arg His Arg Ser Arg Asn Leu Gly
              115                 120
AAA GTC ATC GAC ACC ATT ACG TGT GGT TTT 390
Lys Val Ile Asp Thr Ile Thr Cys Gly Phe
              125                 130
GCC GAC CTC ATG GGG TAC ATC CCT GTC GTT 420
Ala Asp Leu Met Gly Tyr Ile Pro Val Val
              135                 140
GGC GCC CCG GTC GGA GGC GTC GCC AGA GCT 450
Gly Ala Pro Val Gly Gly Val Ala Arg Ala
```

which determined 1..504 description

```
                145                 150
CTG GCA CAC GGT GTT AGG GTC CTG GAA GAT  480
Leu Ala His Gly Val Arg Val Leu Glu Asp
                155                 160
GGG GTA AAT TAT GCA ACA GGG AAT          504
Gly Val Asn Tyr Ala Thr Gly Asn
                165
```

6. Die-Length [of Array Number 6 (1) Array]: -- Mold [of 633 (2) Arrays]: — Number [of Nucleic-Acid (3) Chains]: -- Double Strand (4) Topology: — Class [of Straight Chain-like (5) Array]: -- CDNA to Genomic RNA (6) Origin Living Thing Name: -- HCV (Hepatitis C Virus)
(7) The notation:peptide existence location showing the description:description which is an array : approach:E

(8) 配列

```
TCG ACT ACC ATC TTG GGC ATC GGC ACA GTC  30
Ser Thr Thr Ile Leu Gly Ile Gly Thr Val
                5                  10
CTG GAT CAG GCA GAG ACG GCT GGA GCG CGG  60
Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg
               15                  20
CTC GTC GTG CTC GCC ACC GCC ACG CCT CCG  90
Leu Val Val Leu Ala Thr Ala Thr Pro Pro
               25                  30
GGA TCG ATC ACC GTG CCA CAC CCC AAC ATC  120
Gly Ser Ile Thr Val Pro His Pro Asn Ile
               35                  40
GAG GAA GTG GCC CTG TCC AAC ACT GGG GAG  150
Glu Glu Val Ala Leu Ser Asn Thr Gly Glu
               45                  50
ATT CCC TTC TAT GGC AAA GCC ATC CCC ATT  180
Ile Pro Phe Tyr Gly Lys Ala Ile Pro Ile
               55                  60
GAG GCC ATC AAG GGG GGA AGG CAT CTC ATC  210
Glu Ala Ile Lys Gly Gly Arg His Leu Ile
               65                  70
TTC TGC CAT TCC AAG AAG AAG TGT GAC GAG  240
Phe Cys His Ser Lys Lys Lys Cys Asp Glu
               75                  80
CTC GCC GCA AAG CTG ACA GGC CTC GGA CTC  270
Leu Ala Ala Lys Leu Thr Gly Leu Gly Leu
               85                  90
AAT GCT GTA GCG TAT TAC AGG GGT CTC GAT  300
Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp
               95                 100
GTG TCC GTC ATA CCG ACT AGC GGA GAC GTC  330
Val Ser Val Ile Pro Thr Ser Gly Asp Val
              105                 110
GTT GTC GTG GCA ACA GAC GCT CTA ATG ACG  360
Val Val Val Ala Thr Asp Ala Leu Met Thr
              115                 120
GGT TTT ACC GGC GAC TTT GAC TCA GTG ATC  390
Gly Phe Thr Gly Asp Phe Asp Ser Val Ile
              125                 130
GAC TGC AAC ACA TGT GTC ACC CAG ACA GTC  420
Asp Cys Asn Thr Cys Val Thr Gln Thr Val
              135                 140
GAT TGC AGC TTG GAT CCC ACC TTC ACC ATT  450
Asp Cys Ser Leu Asp Pro Thr Phe Thr Ile
```

which determined 1..633 description

```
                145                 150
GAG ACG ACA ACC GTG CCC CAA GAC GCG GTG  480
Glu Thr Thr Thr Val Pro Gln Asp Ala Val
                155                 160
TCG CGT CCG CAG CGG CGA GGT AGG ACT GGC  510
Ser Arg Pro Gln Arg Arg Gly Arg Thr Gly
                165                 170
AGG GGC AGG AGT GGC ATC TAC AGG TTT GTG  540
Arg Gly Arg Ser Gly Ile Tyr Arg Phe Val
                175                 180
ACT CCA GGA GAA CGG CCC TCA GGC ATG TTC  570
Thr Pro Gly Glu Arg Pro Ser Gly Met Phe
                185                 190
GAC TCC TCG GTC CTG TGT GAG TGC TAT GAC  600
Asp Ser Ser Val Leu Cys Glu Cys Tyr Asp
                195                 200
GCA GGC TGC GCT TGG TAT GAG CTC ACG CCC  630
Ala Gly Cys Ala Trp Tyr Glu Leu Thr Pro
                205                 210
GCT                                     633
Ala
```

7. Die-Length [of Array Number 7 (1) Array]: — Mold [of 582 (2) Arrays]: -- Number [of Nucleic-Acid (3) Chains]: -- Double Strand (4) Topology: — Class [of Straight Chain-like (5) Array]: — CDNA to Genomic RNA (6) Origin Living Thing Name: — HCV (Hepatitis C Virus)
(7) The notation:peptide existence location showing the description:description which is an array : approach:E

(8) 配列

```
GAC CAA ATG TGG AAG TGT CTC ATA CGG CTA  30
Asp Gln Met Trp Lys Cys Leu Ile Arg Leu
                5                  10
AAG CCC ACA CTG CAT GGG CCA ACG CCC CTG  60
Lys Pro Thr Leu His Gly Pro Thr Pro Leu
               15                  20
CTG TAC AGG CTA GGA GCC GTT CAA AAT GAG  90
Leu Tyr Arg Leu Gly Ala Val Gln Asn Glu
               25                  30
GTC ACT CTC ACA CAC CCC ATA ACC AAA TAC  120
Val Thr Leu Thr His Pro Ile Thr Lys Tyr
               35                  40
ATC ATG GCA TGC ATG TCG GCT GAC CTG GAG  150
Ile Met Ala Cys Met Ser Ala Asp Leu Glu
               45                  50
GTC GTC ACT AGC ACC TGG GTG CTA GTA GGC  180
Val Val Thr Ser Thr Trp Val Leu Val Gly
               55                  60
GGA GTC CTT GCG GCT CTG GCC GCG TAC TGC  210
Gly Val Leu Ala Ala Leu Ala Ala Tyr Cys
               65                  70
CTG ACG ACA GGC AGC GTG GTC ATT GTG GGC  240
Leu Thr Thr Gly Ser Val Val Ile Val Gly
               75                  80
AGG ATC ATC TTG TCC GGG AGG CCA GCT GTT  270
Arg Ile Ile Leu Ser Gly Arg Pro Ala Val
               85                  90
ATT CCC GAC AGG GAA GTC CTC TAC CAG GAG  300
Ile Pro Asp Arg Glu Val Leu Tyr Gln Glu
               95                 100
TTC GAT GAG ATG GAA GAG TGT GCT TCA CAC  330
Phe Asp Glu Met Glu Glu Cys Ala Ser His
              105                 110
CTC CCT TAC ATC GAG CAA GGA ATG CAG CTC  360
Leu Pro Tyr Ile Glu Gln Gly Met Gln Leu
              115                 120
GCC GAG CAA TTC AAA CAG AAG GCG CTC GGA  390
Ala Glu Gln Phe Lys Gln Lys Ala Leu Gly
              125                 130
TTG CTG CAA ACA GCC ACC AAG CAA GCG GAG  420
Leu Leu Gln Thr Ala Thr Lys Gln Ala Glu
              135                 140
GCT GCT GCT CCC GTG GTG GAG TCC AAG TGG  450
Ala Ala Ala Pro Val Val Glu Ser Lys Trp
```

which determined 1..582 description

```
                145                 150
CGA GCC CTT GAG GTC TTC TGG GCG AAA CAC  480
Arg Ala Leu Glu Val Phe Trp Ala Lys His
                155                 160
ATG TGG AAC TTC ATC AGC GGG ATA CAG TAC  510
Met Trp Asn Phe Ile Ser Gly Ile Gln Tyr
                165                 170
TTG GCA GGC CTA TCC ACT CTG CCT GGA AAC  540
Leu Ala Gly Leu Ser Thr Leu Pro Gly Asn
                175                 180
CCC GCG ATA GCA TCA TTG ATG GCT TTT ACA  570
Pro Ala Ile Ala Ser Leu Met Ala Phe Thr
                185                 190
GCC TCT ATC ACC                         582
Ala Ser Ile Thr
```

8. Die-Length [of Array Number 8 (1) Array]: — Mold [of 480 (2) Arrays]: — Number [of Nucleic-Acid (3) Chains]: — Double Strand (4) Topology: — Class [of Straight Chain-like (5) Array]: — CDNA to Genomic RNA (6) Origin Living Thing Name: — HCV (Hepatitis C Virus)
(7) The notation:peptide existence location showing the description:description which is an array : approach:E

(8) 配列

```
AAA TGC CCA TGC CAG GTT CCG GCC CCC GAA  30
Lys Cys Pro Cys Gln Val Pro Ala Pro Glu
                5                  10
TTT TTC ACG GAG GTG GAT GGA GTA CGG TTG  60
Phe Phe Thr Glu Val Asp Gly Val Arg Leu
               15                  20
CAC AGG TAT GCT CCG GTG TGC AAA CCT CTC  90
His Arg Tyr Ala Pro Val Cys Lys Pro Leu
               25                  30
CTA CGA GAG GAG GTC GTA TTC CAG GTC GGG 120
Leu Arg Glu Glu Val Val Phe Gln Val Gly
               35                  40
CTC AAC CAG TAC CTG GTC GGG TCA CAG CTC 150
Leu Asn Gln Tyr Leu Val Gly Ser Gln Leu
               45                  50
CCA TGT GAA CCC GAA CCG GAC GTA GCA GTG 180
Pro Cys Glu Pro Glu Pro Asp Val Ala Val
               55                  60
CTC ACT TCC ATG CTC ACC GAC CCC TCT CAT 210
Leu Thr Ser Met Leu Thr Asp Pro Ser His
               65                  70
ATT ACA GCA GAG ATG GCC AAG CGT AGG CTG 240
Ile Thr Ala Glu Met Ala Lys Arg Arg Leu
               75                  80
GCC AGG GGG TCT CCC CCC TCC TTG GCC AGC 270
Ala Arg Gly Ser Pro Pro Ser Leu Ala Ser
               85                  90
TCT TCA GCT AGC CAG TTG TCT GCG CCT TCT 300
Ser Ser Ala Ser Gln Leu Ser Ala Pro Ser
               95                 100
TTG AAG GCG ACA TGT ACT ACC CAT CAT GAC 330
Leu Lys Ala Thr Cys Thr Thr His His Asp
              105                 110
TCC CCG GAC GCT GAC CTC ATC GAG GCC AAC 360
Ser Pro Asp Ala Asp Leu Ile Glu Ala Asn
              115                 120
CTC CTG TGG CGG CAG GAG ATG GGC GGG AAC 390
Leu Leu Trp Arg Gln Glu Met Gly Gly Asn
              125                 130
ATC ACC CGA GTG GAG TCA GAA AAT AAG GTG 420
Ile Thr Arg Val Glu Ser Glu Asn Lys Val
              135                 140
GTA ATC CTG GAC TCT TTC GAT CCG ATT CGG 450
Val Ile Leu Asp Ser Phe Asp Pro Ile Arg
              145                 150
GCG GTG GAG GAT GAG AGG GAA GTA TCC GTT 480
Ala Val Glu Asp Glu Arg Glu Val Ser Val
              155                 160
```

which determined 1..480 description

[Translation done.]

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(54)【発明の名称】Ｃ型肝炎診断用免疫学的凝集反応試薬

(57)【要約】

【構成】　加熱処理されたＣ型肝炎ウイルス由来遺伝子のＨＣＶ抗原活性ポリペプチドを抗原として使用することを特徴とするＣ型肝炎診断用免疫学的凝集反応試薬。

【効果】　このＣ型肝炎診断用免疫学的凝集反応試薬は、検出感度及び特異性が極めて優れており、且つ短時間に判定ができる。

1

【特許請求の範囲】

【請求項1】　加熱処理されたC型肝炎ウイルス由来遺伝子のHCV抗原活性ポリペプチドを抗原として使用することを特徴とするC型肝炎診断用免疫学的凝集反応試薬。

【請求項2】　HCV抗原活性ポリペプチドが配列番号1のアミノ酸配列を含むHCV抗原活性ポリペプチド、配列番号2のアミノ酸配列を含むHCV抗原活性ポリペプチド、配列番号3のアミノ酸配列を含むHCV抗原活性ポリペプチド及び配列番号4のアミノ酸配列を含むHCV抗原活性ポリペプチドを含むことを特徴とする請求項1の免疫学的凝集反応試薬。

【請求項3】　配列番号1のアミノ酸配列を含むHCV抗原活性ポリペプチド、配列番号2のアミノ酸配列を含むHCV抗原活性ポリペプチド、配列番号3のアミノ酸配列を含むHCV抗原活性ポリペプチド及び配列番号4のアミノ酸配列を含むHCV抗原活性ポリペプチドを不溶性担体粒子に担持してなることを特徴とするC型肝炎診断用免疫学的凝集反応試薬。

【発明の詳細な説明】

【0001】

【産業上の利用分野】本発明は、C型肝炎の病因であるC型肝炎ウイルス（以下、HCVとも略記する）に対する抗体を検出するためのC型肝炎診断用免疫学的凝集反応試薬に関する。

【0002】

【従来技術】C型肝炎はHCVにより引き起こされるものであり、輸血後非A非B型慢性肝炎の9割以上はHCVの感染により引き起こされる肝炎と言われている。HCVの遺伝子の一部がヨーロッパ特許EP0318216（1989年公開）、及びヨーロッパ特許EP0388232（1990年公開）に報告されている。

【0003】これまでの研究によると、HCVは遺伝子配列全長約10kb（約1万ヌクレオチド）のRNAウイルスと考えられている。HCV遺伝子から生産される蛋白のうち、抗原活性の高いポリペプチドを抗原としてC型肝炎診断用試薬が開発されてきた。例えば、ヨーロッパ特許0318216では非構造蛋白領域をコードする遺伝子の一部を酵母の発現ベクターに挿入し、この遺伝子を発現させて、C100と呼ばれるHCV抗原活性ポリペプチドを抗原に用いたC型肝炎診断用試薬もその一つである。

【0004】C型肝炎診断には現在、1種あるいは2種のHCV抗原活性ポリペプチドを抗原として用いた酵素免疫法（以下、EIA法とも略す）及び受身凝集反応（以下、PA法とも略す）による検査法が用いられている。上記診断薬は輸血時におけるC型肝炎の予防に効果をあげている。しかしながら、1種及び2種のHCV原活性ポリペプチドを抗原として用いているため、偽陰性、偽陽性が多く且つHCV感染初期の診断の感度に問題があるのが現状である。

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【0005】

【発明が解決しようとする課題】現在用いられているC型肝炎診断薬は輸血時のC型肝炎ウイルスの感染の予防に大きな効果をあげているが、より感度及び特異性の高く且つHCV感染初期に診断可能な診断薬の開発が望まれている。本発明者はこの課題を解決すべく鋭意研究を行い、その結果、加熱したC型肝炎ウイルス遺伝子由来のHCV抗原活性ポリペプチドを抗原として使用することにより診断の感度及び特異性の優れたC型肝炎診断用免疫学的凝集反応試薬が得られることを見い出し、本発明を完成するに到った。

【0006】

【課題を解決するための手段】本発明は、加熱処理されたC型肝炎ウイルス由来遺伝子のHCV抗原活性ポリペプチドを抗原として使用することを特徴とするC型肝炎診断用免疫学的凝集反応試薬にある。上記HCV抗原活性ポリペプチドは、配列番号1のアミノ酸配列を含むHCV抗原活性ポリペプチド、配列番号2のアミノ酸配列を含むHCV抗原活性ポリペプチド、配列番号3のアミノ酸配列を含むHCV抗原活性ポリペプチド、配列番号4のアミノ酸配列を含むHCV抗原活性ポリペプチドを含むものである。

【0007】さらに、本発明は、配列番号1のアミノ酸配列を含むHCV抗原活性ポリペプチド、配列番号2のアミノ酸配列を含むHCV抗原活性ポリペプチド、配列番号3のアミノ酸配列を含むHCV抗原活性ポリペプチド及び配列番号4のアミノ酸配列を含むHCV抗原活性ポリペプチドを不溶性担体粒子に担持してなるC型肝炎診断用免疫学的凝集反応試薬にある。

【0008】以下に本発明を詳細に説明する。本発明における加熱処理とは、ポリペプチドの混合物を所定の温度に加熱することにより行うものである。この加熱温度は20℃以上80℃以下が有効である。好ましくは25℃以上60℃以下であり、さらに好ましくは35℃以上50℃以下である。ポリペプチドの加熱処理は、緩衝作用のある緩衝液中で行い、その種類はいずれでもよい。例えば、燐酸緩衝液、グリシン緩衝液、トリス緩衝液、酢酸緩衝液等々である。pHについてもいずれでもよいが中性領域が望ましい。好ましくは燐酸緩衝液、pH6.0から8.0が望ましい。処理時間は10分間以上であれば、いずれでもよく、好ましくは10分間から5時間以下、さらに好ましくは30分間以上2時間以下である。

【0009】本発明で言うC型肝炎ウイルス遺伝子は、例えば、Proc. Natl. Acad. Sci. USA, Vol. 87, pp. 9524 ～9528 (1990)に記載されている塩基配列を有する全長約10kb（約1万ヌクレオチド）のRNAである。HCVはRNAウイルスであるが、HCV由来のRNAより逆転写酵素により作り出されたcDNAも該C型肝炎ウイルス遺伝子に該当する。

【００１０】該Ｃ型肝炎ウイルス遺伝子は、輸血後非Ａ非Ｂ肝炎患者の血清からウイルス遺伝子を分離して作製したｃＤＮＡライブラリーから得ることが出来る。例えば、まず患者血清から超遠心によりＣ型肝炎ウイルスを分離し、次いでウイルスから遺伝子ＲＮＡを調製し、該ＲＮＡに対して逆転写酵素を使用してｃＤＮＡを合成し、しかるのちに該ｃＤＮＡ断片をプラスミドベクターあるいはファージベクターに挿入して、ｃＤＮＡライブラリーを調製する。次いで、該ｃＤＮＡライブラリーを、輸血後非Ａ非Ｂ肝炎患者の血清（抗ＨＣＶ抗体を含有する血清）を用いイムノスクリーニングすることにより、目的の遺伝子を得ることができる。また公知のＨＣＶ遺伝子の塩基配列をもとにＤＮＡプローブを合成して、ｃＤＮＡライブラリーをＤＮＡ／ＤＮＡハイブリダイゼーションによりスクリーニングしてもよい。また別の方法としては Proceedings of the Japan Academy, Vol. 65, Ser. B, No. 9, pp. 219～223 (1989). に示される方法、即ち逆転写酵素とＰＣＲ法とを組み合わせたＲＴ－ＰＣＲ法により目標の領域を遺伝子増幅させて、その増幅させた遺伝子断片をクローニングする方法も有効である。

【００１１】本発明におけるＨＣＶ抗原活性ポリペプチドはＣ型肝炎患者血清及び血漿に含まれる抗ＨＣＶ抗体と免疫学的反応性を有する。すなわち、抗ＨＣＶ抗体に対するエピトープ部位を有し、抗原抗体反応によりＣ型肝炎患者血清及び血漿中の抗ＨＣＶ抗体と特異的に結合する特性を有する。該ＨＣＶ抗原活性ポリペプチドはＣ型肝炎診断用試薬の抗原として用いることが可能である。

【００１２】該ＨＣＶ抗原活性ポリペプチドはＨＣＶの遺伝子により生産されるポリペプチドである。さらにＨＣＶ抗原活性ポリペプチドの長さはいずれでもよく、好ましくは３アミノ酸残基以上3000アミノ酸残基以下、さらに好ましくは３アミノ酸残基以上2000アミノ酸残基以下である。該ＨＣＶ抗原活性ポリペプチドは、通常知られている遺伝子発現系、即ち、大腸菌のホスト・ベクター系、枯草菌のホスト・ベクター系、酵母のホスト・ベクター系、昆虫細胞あるいは昆虫のホスト・ベクター系、動物細胞のホスト・ベクター系等を利用して発現が可能である。このうち、大腸菌は好適に利用できる。大腸菌を用いて該ＨＣＶ抗原活性ポリペプチドを発現するには、まず大腸菌で発現可能なベクターにＨＣＶの遺伝子を挿入し組換えベクターを作製する。ベクターは特に限定されず、大腸菌のベクターとして通常用いられるベクターならば如何なるベクターでも利用できるが、特に遺伝子発現が高頻度で起こるベクターは好適に利用される。例えば、一連のｐＵＣベクター（宝酒造（株）製品）、一連のｐＴＶベクター（宝酒造（株）製品）、一連のｐＴＺベクター（東洋紡績（株）製品）、一連のｐＥＴ（Methods in enzymology, Vol. 185に示される）などが利用できる。また、一連のｐＵＥＸベクター（アマシャム・ジャパン（株）製品）、一連のｐＥＸベクター（ベーリンガー・マンハイム山之内（株）製品）を利用すれば、ＨＣＶ抗原活性ポリペプチドをβ－ガラクトシダーゼとの融合ポリペプチドとして発現させることができる。大腸菌で発現可能なベクターには、通常は大腸菌内で働く遺伝子発現のためのプロモーターや、それをコントロールするオペレーターが附属している。このようなベクターのプロモーターの下流にある適当な制限酵素部位を利用してＨＣＶ遺伝子を挿入することにより、組換えベクターが作製される。組換えベクターにより大腸菌を形質転換し、該形質転換大腸菌を培養し挿入されたＨＣＶ遺伝子を発現させることによりＨＣＶ抗原活性ポリペプチドが生産される。

【００１３】組換えベクターで遺伝子発現を行う場合は、ポリペプチドのＮ末端あるいはＣ末端にランダムな配列のアミノ酸が複数個付加する場合がある。しかしながら、このようなＮ末端、あるいはＣ末端に付加された複数個のアミノ酸はランダムなアミノ酸であるから、ＨＣＶ抗原活性には無関係であり、抗原活性測定には影響しない。

【００１４】該ＨＣＶ抗原活性ポリペプチドは、上記形質転換大腸菌を培養し得られた菌体を超音波処理などの方法で破砕し、この菌体破砕物より公知の方法により分離される。該ＨＣＶ抗原活性ポリペプチドの精製方法は公知の方法ならばいずれでもよく塩析、イオン交換樹脂吸着、ゲル濾過等々である。好ましくは上記方法の組合せが有効である。また、精製された該ＨＣＶ抗原活性ポリペプチドはどの様な溶液に分散されていてもよいが、好ましくは0.87％塩化ナトリウム水溶液（以下、生理食塩水とも略記する）あるいは0.87％塩化ナトリウム含有、20mM燐酸緩衝液、ｐＨ7.2（以下、ＰＢＳとも略記する）に分散されていることが望ましい。また、精製純度としては高い方がよい。好ましくは該ＨＣＶ抗原活性ポリペプチドが全蛋白質中の80％以上が望ましい。

【００１５】本発明でいう第１のＨＣＶ抗原活性ポリペプチドとは、優れた抗原活性に必須な、配列番号１に示すアミノ酸配列を含むポリペプチド（以下、Ｃｏｒｅ抗原とも略記する）であり、そのＮ末端あるいはＣ末端に、余分なアミノ酸が複数個付加したものであってもよい。該配列は日本型ＨＣＶのＮ末端から１番目ないし１６８番目までのコア蛋白質のアミノ酸配列に相当する。

【００１６】本発明でいう第２のＨＣＶ抗原活性ポリペプチドとは、優れた抗原活性に必須な、配列番号２に示すアミノ酸配列を含むポリペプチド（以下、ＮＳ－３抗原とも略記する）であり、そのＮ末端あるいはＣ末端に、余分なアミノ酸が複数個付加したものであってもよい。この配列の１番目から211番目までは、日本型ＨＣＶのＮ末端から数えて、1323番目から1533番目までのＮＳ－３蛋白質のアミノ酸に相当する。

【００１７】本発明でいう第３のＨＣＶ抗原活性ポリペプチドとは、優れた抗原活性に必須な、配列番号３に示すアミノ酸配列を含むポリペプチド（以下、ＮＳ－４抗原とも略記する）であり、そのＮ末端あるいはＣ末端に、余分なアミノ酸が複数個付加したものであってもよい。この配列の１番目から194番目までは、日本型ＨＣＶのＮ末端から数えて、1605番目から1798番目までのＮＳ－４蛋白質のアミノ酸に相当する。

【００１８】本発明でいう第４のＨＣＶ抗原活性ポリペプチドとは、優れた抗原活性に必須な、配列番号４に示すアミノ酸配列を含むポリペプチド（以下、ＮＳ－５抗原とも略記する）であり、そのＮ末端あるいはＣ末端に、余分なアミノ酸が複数個付加したものであってもよい。この配列の１番目から160番目までは、日本型ＨＣＶのＮ末端から数えて、2111番目から2270番目までのＮＳ－５蛋白質のアミノ酸に相当する。

【００１９】本発明における不溶性担体粒子としては公知の凝集法の診断薬に用いることができる担体ならば何でもよく、例えば、核部となる無機質化合物に染料を被覆させた高比重複合粒子（特開昭62-115366号、以下、ＨＤＰとも略記する）、羊赤血球、ポリスチレン粒子、ゼラチン粒子等である。好ましくはＨＤＰ、羊赤血球、ポリスチレンが用いられる。さらに好ましくはＨＤＰである。また、本発明で用いる不溶性担体の粒子径も凝集法診断試薬として用いる範囲のものならば、いずれでもよく、好ましくは0.01μｍから20μｍまでの粒子径のものであり、さらに好ましくは0.01μｍから３μｍのものである。また、不溶性担体の比重もいずれのものでもよく、好ましくは1.0から2.5である。

【００２０】本発明でいう担持とは、不溶性担体にＨＣＶ抗原活性ポリペプチドを吸着させる方法で公知の吸着される方法ならばいずれでもよく、物理的吸着法、化学的吸着法等々いずれでもよい。例えば、疎水的吸着、塩化クロム法等々である。好ましくは疎水的吸着法が望ましい。前記担持は緩衝作用のある緩衝液中で行い、その種類はいずれでもよい。例えば、燐酸緩衝液、グリシン緩衝液、トリス緩衝液、酢酸緩衝液等々である。ｐＨについてもいずれでもよいが中性領域が望ましい。好ましくは燐酸緩衝液、ｐＨ6.0から8.0が望ましい。

【００２１】ＨＣＶ抗原活性ポリペプチドを担体に担持させる場合は、蛋白質濃度には特に限定はないが好ましくは0.1μｇ／ｍｌ以上が適当である。また、不溶性担体粒子に担持させる時間及び温度には特に限定されないが、温度は好ましくは１℃以上80℃以下、時間は30分間以上で、好適に行うことが出来る。本発明のＣ型肝炎診断用免疫学的凝集反応試薬は、水性懸濁液の状態で使用されるが、長期の保存においてはこれを凍結乾燥することが好ましい。本発明の凝集反応用試薬は、かかる凍結乾燥後、再び水性懸濁液としても前記した保存時の安定性及び反応時の凝集像の切れが低下することなく、優れた性能を示す。　上記凍結乾燥方法は限定的ではなく通常の方法で行えばよい。例えば感作赤血球の凍結乾燥法に採用される方法及び条件が用いられる。好ましくは急速予備凍結し次いで真空凍結乾燥する方法が採用される。該急速予備凍結には液体窒素、ドライアイス- メタノール、ドライアイス- アセトンあるいはフルオロカーボン等に、上記水性懸濁液の入ったバイアル又はアンプル等の容器を浸漬することにより達成される。

【００２２】また、真空凍結乾燥方法は、一般には、上記感作担体の浮遊液の入ったバイアル等を急速予備凍結したのち、予め－40～－60℃に冷却した凍結乾燥機のチャンバー内に置き24～72時間かけて徐々に昇温し真空凍結乾燥する方法が好適である。この時のチャンバー内の圧力50～200μＨｇ、最終乾燥温度は20～50℃が適当である。ついで真空状態、または不活化ガスを充填して封栓保存すればよい。但し、真空凍結乾燥方法は前記方法に限定されるものではない。

【００２３】本発明の凝集反応用試薬は、通常診断に利用される凝集反応法が何ら制限なく適用される。例えば、定性診断の平板法、半定量診断のマイクロタイター法及び定量診断の比濁法、粒子数計測法等である。そのうち、特にマイクロタイター法に適用する場合、本発明の効果が特に顕著である。本発明でいうＣ型肝炎診断用免疫学的凝集反応試薬とはＣ型肝炎患者の血清または血漿中に存在する抗ＨＣＶ抗体を免疫学的凝集反応で検出することによりＣ型肝炎の診断を行う診断用試薬である。通常診断に利用される凝集反応法が何ら制限なく適用される。例えば、定性診断の平板法、半定量診断のマイクロタイター法及び定量診断の比濁法、粒子数計測法等である。そのうち、特にマイクロタイター法に適用する場合、本発明の効果が特に顕著である。

【００２４】

【発明の効果】本発明のＣ型肝炎診断用免疫学的凝集反応試薬は従来品に比べて検出感度及び特異性が著しく優れており、且つ短時間で判定が可能である。また、凝集反応の判定の基準である抗原抗体反応による凝集形成物（以下、管底凝集像とも略記する）が極めて明確に形成される。従って、本発明のＣ型肝炎診断用免疫学的凝集反応試薬は従来のものに比較して極めて優れたＣ型肝炎診断試薬である。

【００２５】

【実施例】以下に実施例及び比較例を挙げて本発明をさらに具体的に説明する。但し、これらの実施例により本発明の技術的範囲が限定されるものではない。本実施例では特に断わらない限り、遺伝子操作実験の手法は、サムブロックらの方法［Sambrook, J., Fritsch, E. F., Maniatis, T., Molecular Cloning, 2nd ed., Cold Spring Harbor Laboratory Press, New York (1989).］に従って行った。なお、制限酵素は宝酒造（株）製品を使用した。

【００２６】（実施例１）　Ｃｏｒｅ抗原の製造

（１－１）組換えプラスミドpGC03 の作製

（ＲＮＡの調製）輸血後非Ａ非Ｂ肝炎患者血清3000mlを19,000rpm で16時間超遠心し、沈澱を得た。該沈澱物をＧＩＴＣ溶液（4Mグアニジウムイソチオシアネート（フルカ（株）製），25mMクエン酸ソーダ，0.5 %サルコシル，0.1Mメルカプトエタノール）100ml に溶解し、該溶解物100ml に対して、100ml のフェノールークロロホルム（1：1）を加え、15分間室温で振盪後、3000rpm 、15分間遠心した。該反応液の水層を取り出し、イソプロピルアルコール100 mlを加え、－20℃に３時間放置した後、3000rpm で15分間遠心し、沈澱物を得た。

【００２７】該沈澱物に対して、ＧＩＴＣ溶液10mlを加えて溶解液とした。該溶解液に対して、10mlのフェノールークロロホルム（1：1）を加え、10分間室温で振盪後、3000rpm, 15 分間遠心した。該反応液の水層を取り出し、クロロホルム20mlを加え、５分間振盪した。振盪後、3000rpm で５分間遠心し、水層10mlを回収した。この水層10mlに対して、5M NaCl 溶液 0.4mlを加えた。

【００２８】その後、30mlの氷冷エタノールを添加し、－20℃で12時間放置した。放置後、3000rpm で15分間遠心し、沈澱物を得た。該沈澱物を75%エタノールで洗浄し、乾燥後、蒸留水200 μl に溶解し、ＲＮＡ溶液を得た。

（ｃＤＮＡライブラリーの構築）ｃＤＮＡ合成はＢＲＬ社の合成キットを使用した。その方法はｃＤＮＡ合成マニュアル［ＢＲＬ／コスモバイオ社 Instruction Manual, Cat. No8267SA］に従って行った。本実施例の（ＲＮＡの調製）の項で、非Ａ非Ｂ患者血清より調製した１本鎖ＲＮＡ溶液5 μl にランダムプライマー溶液(100μM)［宝酒造（株）製品，製品カタログ番号3810］を5 μl 加え、逆転写酵素反応を行い、ＲＮＡ／ＤＮＡの２本鎖とした。次いで大腸菌ＤＮＡポリメラーゼＩと、大腸菌ＲＮＡ分解酵素Ｈとを加え、ＤＮＡ／ＤＮＡ２本鎖とした。

【００２９】次に、こうして得られた２本鎖ＤＮＡの両末端にEcoRI リンカーを結合させた。この処理には宝酒造の酵素を用い、宝酒造の酵素に添付されている反応条件で反応を行った。まず２本鎖ＤＮＡ約1 μg を用いて、EcoRI メチラーゼ処理を行い、その後T4 ＤＮＡリガーゼ反応によりEcoRI リンカー(dGGAATTCC) を結合させた。最後に得られた反応液をEcoRI で切断し、EcoRI 断片を回収した。

【００３０】最後にこのEcoRI 断片をλgt11のEcoRI 部位に挿入し、組換えλgt11ファージを作製したが、これにはStratagene社のキットGIGAPACKII GOLD を用い、方法はキットに添付されているマニュアル［Protocol/Instruction Manual Cat. #200214, 200215, 200216, December 6, 1989］に従った。まずλgt11のEcoRI 部位にEcoRI 断片を挿入し、これをT4 ＤＮＡリガーゼにより結合させた。得られた組換えファージＤＮＡ溶液をGIGAPACKII GOLD のIn Vitro Packaging Kitを用いて、ファージに戻した。この時のタイターを滴定したところ1.0 × 10^6 であった。このタイター値は、独立したクローンの数を示す。

（イムノスクリーニング）λgt11に挿入されたｃＤＮＡはフレームが一致すると、λgt11に組み込まれているβ－ガラクトシダーゼとの融合蛋白として、ｃＤＮＡがコードしているアミノ酸配列が表現される。この融合蛋白を非Ａ非Ｂ型肝炎患者血清を大腸菌の菌体で吸収したものでスクリーニングした。指示菌はE. coli Y1090を使用した。直径15cmのL-bottom plate（水1 リットル当りBacto-tryptone 10g, NaCl 5g, Yeastextract 5g, Bacto-agar 15g を加え、オートクレーブ滅菌）に１枚のプレート当りプラークが約４万個となるように調製したファージ液とY1090 を37℃で15分インキュベートした。それに45℃に温めておいた0.7 %L-top agarose 2.5ml を混合し、L-bottom plateにひろげ、固化後42℃で3.5 時間インキュベートした。一方ニトロセルロースフィルターを10mMイソプロピルチオーβ－D －ガラクトシド（ＩＰＴＧ）溶液に数分間ひたした後、室温で乾燥した。該フィルターを上該プレートにのせ、37℃で一夜インキュベートした。インキュベート後、フィルターをはがし、ＴＮＴ緩衝液（10mMトリス－HCl (pH8.0), 150mM NaCl, 0.05%Tween20 ）にひたし、よくリンスした。再度、新しいＴＮＴ緩衝液に振盪しながら、30分間ひたした。さらに該フィルターをブロッキング緩衝液（20%牛胎児血清含有ＴＮＴ緩衝液）で30分間インキュベートした。次ぎにフィルターをブロッキング緩衝液で150 倍希釈した一次抗体液（非Ａ非Ｂ型肝炎患者プール血清をY1090の超音波破砕液で吸収したもの）と室温で４時間ゆっくり振盪しながら反応させた。次いでフィルターを0.1 %牛血清アルブミン（ＢＳＡ）含有ＴＮＴ緩衝液、0.1 %ＢＳＡ+0.1 %NP-40 含有ＴＮＴ緩衝液、0.1 %ＢＳＡ含有ＴＮＴ緩衝液の順で10分間ずつ洗浄した。次ぎに、10μl の西洋ワサビペルオキシダーゼ標識抗ヒトＩｇＧヤギＩｇＧ（Kirkeguard & Perry Lab社製）を含有する15mlのブロッキング緩衝液にフィルターをひたし、室温で２時間反応させた後、0.1 %ＢＳＡ含有ＴＮＴ緩衝液、0.1 %ＢＳＡ+0.1 %NP-40 含有ＴＮＴ緩衝液で10分間ずつ洗浄した。さらにフィルターを10mMトリス－HCl (pH7.5), 150mM NaClで１分間洗浄後、染色液［60mg 4－クロローナフトールを含むメタノール20mlを使用直前に、30 %H_2O_2 の60μl を含む10mMトリス－HCl (pH7.5), 150 mM NaCl溶液100mlと混合したもの］に室温で15分反応し、２回蒸留水で洗浄した後、紫色に発色した陽性プラークを得た。

【００３１】この組換えファージからファージＤＮＡを調製し、EcoRI で処理して、ｃＤＮＡの断片をアガロース電気泳動ゲルから回収し、プラスミドベクターpUC18

のEcoRI 部位に挿入した。該プラスミドをpGC03 と命名し、塩基配列を決定した。このｃＤＮＡ断片には、ＨＣＶの構造蛋白質遺伝子のコア領域が含まれていることが明かとなった。

（１－２）大腸菌HB101 ［pHCX01］の作製

pGC03 をHinfI で消化後、ＤＮＡポリメラーゼＩ Klenow fragmentにより末端を平滑化した。このＤＮＡとBamHＩ リンカー（dCGGATCCG, 宝酒造（株）製）をT4 ＤＮＡリガーゼにより連結反応を行い、更にBamHI で消化し、アガロース電気泳動ゲルからコア領域を含む0.56kb 断片を回収した。この0.56kb断片をプラスミドベクターpUC19 のBamHI 部位に挿入し、更に該プラスミドをBspHＩ (New England Biolabs 社製品）で消化後、T4 ＤＮＡポリメラーゼにより末端を平滑化した。このＤＮＡをBamHI で消化し、アガロース電気泳動ゲルから5′側非翻訳領域を除いた0.51kbのコア領域ＤＮＡ断片を回収した。この0.51kb断片をプラスミドベクターpUEX2 (Amersham社製）のSmaI～BamHI 部位に挿入して、組換えベクターpHCX01を得た。得られたpHCX01について、プラスミド法による塩基配列の決定〔服部らの方法、Anal. Biochem., Vol.152, pp.232～238(1986) をおこなった。この組換えベクターpHCX01には、ＨＣＶのN末端から１番目ないし１６８番目のアミノ酸配列をコードする塩基配列が含まれ、その塩基配列は配列番号５に示すとおりである。次に、組換えベクターpHCX01で宿主大腸菌HB101 を形質転換し、組換え大腸菌HB101 ［pHCX01］を得た。組換え大腸菌HB101 ［pHCX01］は、茨城県つくば市東１丁目１番３号の通商産業省工業技術院微生物工業技術研究所に微工研菌寄第１３０５６号として寄託されている。この組換え大腸菌HB101 ［pHCX01］をＬＢ＋Amp培地［Bacto tryptone 1.0%, Yeast extract 0.5%, NaCl0.5%, アンピシリン(Amp)50 μg ／ml］で30℃で一晩培養し、最終濃度が15％となるようにグリセリンを添加して－80℃で凍結保存した。

（１－３）Ｃｏｒｅ抗原の製造

組換え大腸菌HB101 ［pHCX01］を培養し遺伝子発現を行うことにより、Ｃｏｒｅ抗原はβ－ガラクトシダーゼとの融合ポリペプチドとして生産される。組換え大腸菌HB101 ［pHCX01］の凍結保存菌体１mlを、１リットルのＬＢ＋Amp培地に接種し30℃にて一晩培養した。続いてこの培養物を、20リットルのＬＢ＋Amp培地に植菌し30℃でOD540 が1.5 となるまで培養し、培養温度を42℃に上昇させて引き続き３時間培養した。培養後、遠心分離により集菌し57g の湿菌体を得た。菌体を２リットルの、0.6M尿素を含むTNE 緩衝液（50mM Tris・HCl(pH8.3), 100mM NaCl, 1mM EDTA) に懸濁し、超音波処理により破砕した。この菌体破砕物を10,000g 、20分間の遠心分離により、Ｃｏｒｅ抗原を含む不溶性顆粒を沈澱画分に回収した。この沈澱を、再び２リットルの0.6M尿素を含むTNE 緩衝液に懸濁して不溶性顆粒を洗浄し、遠心分離することにより沈澱を回収した。更にこの沈澱を、２リットルの3M尿素を含むTNE 緩衝液に懸濁し、室温で30分間撹はんすることにより不溶性顆粒を十分洗浄した後、遠心分離することにより不溶性顆粒を沈澱画分に回収した。この不溶性顆粒の沈澱に、200ml の8M尿素を含むTNE緩衝液を加え沈澱を可溶化した。これを16,000g 、20分間の遠心分離により上清を分取し、TNE 緩衝液に対して透析した。透析後、16,000g 、20分間の遠心分離により上清を分取しＣｏｒｅ抗原を得た。20リットルの培養液から980mg のＣｏｒｅ抗原が得られた。得られたＣｏｒｅ抗原について、ＳＤＳポリアクリルアミド電気泳動（ＳＤＳ－ＰＡＧＥ）により分子量を調べ、そのアミノ酸配列より計算される分子量（１３７ｋｄ）と一致することを確認した。

【００３２】（実施例２）　ＮＳ－３抗原の製造

（２－１）組換えプラスミドpHCV7 の作製

優れた抗原活性を示すことが予想されるＨＣＶのＮＳ３領域の遺伝子断片について、その断片の両側20塩基ずつのプライマーをセットとして用い、ＲＴ－ＰＣＲ法による遺伝子増幅を行った。プライマーは、アプライドバイオシステムズ社製品、３４０Ａ型機を用いて合成した。なお、５’上流側プライマーの塩基配列は、（５’）ＣＣＧＡＣＧＧＴＧＧＡＴＧＣＴＣＣＧＧＧ（３’）、３’下流側プライマーの塩基配列は、（５’）ＣＴＧＧＡＧＣＣＡＡＴＣＣＡＡＣＧＣＣＣ（３’）である。

【００３３】まず、実施例１で得られたＲＮＡ溶液4 μl に、逆転写酵素反応液［250mM Tris・HCl (pH8.3), 375mM KCl, 50mM DTT, 15mM MgCl2] 2 μl 、３’下流側のアンチセンス鎖プライマー溶液(25ng/μl)1 μl 、４種類のデオキシヌクレオチド［dATP, dGTP, dCTP, dTTP、各15mM］を各0.5 μl ずつ加えて、9 μl の溶液を作った。これにミネラルオイルを加えて、70℃、２分間加熱し、ついで37℃に冷却し、逆転写酵素1 μl （ＢＲＬ社製品）を加え、37℃で60分反応させた。この反応液(10 μl)に、更にＰＣＲ反応液［400mM Tris・HCl(pH8.8), 100mM 硫酸アンモニウム, 40mM 塩化マグネシウム, 60mM メルカプトエタノール, 0.1％ BSA] 8.3 μl 、４種類のデオキシヌクレオチド［dATP, dGTP, dCTP, dTTP, 各15mM］を各5 μl ずつ加えた。次いで、遺伝子増幅させる目的の領域をはさんで、５’上流側のセンス鎖の塩基配列を持つ20塩基のプライマー溶液(100ng／μl)5μl と、更に３’下流側のアンチセンス鎖の塩基配列を持つ20塩基のプライマー溶液5 μl (100ng／μl)を加え、最後に水0.7 μl を加え、全量49μl の溶液とした。この溶液を92℃で５分間処理し、室温に冷却してTaq ポリメラーゼ1 μl （２単位、New England Biolabs 社製品）を加えた。以下、アニール(55 ℃、45秒)、ポリメリゼーション(72 ℃、２分)、変性(90 ℃、１分) を、35回繰り返して、ＤＮＡの増幅を行った。

【００３４】ＲＴ－ＰＣＲ法により増幅した遺伝子産物

のH7 断片を、アガロースゲル（２%）で電気泳動し目的の長さのＤＮＡを回収した。ついでこれをKlenow fragment 酵素処理し、ＤＮＡの末端を平滑に揃え、更にT4 ポリヌクレオチドキナーゼにより、５’末端をリン酸化した。これをプラスミドベクターpTZ19RのHincII部位に挿入し、遺伝子のクローン化を行った。こうして組換えプラスミドpHCV7 を得た。

【００３５】組換えプラスミドpHCV7 により形質転換された大腸菌は、E. coli HCV7と表示し、通商産業省工業技術院微生物工業技術研究所に微工研菌寄第11831 号として寄託されている。

（２－２）大腸菌HB101 ［pCI07 ］の作製

pHCV7 をEcoRI とStuIで消化し、ｃＤＮＡの５’側の338bp 断片を得た。この338bp 断片はさらにHinfI で部分消化後、ＤＮＡポリメラーゼI Klenow fragmentにより末端を平滑化し、263bp 断片を得た。またpHCV7 をStuI で消化し、CIP処理した後、PstI消化し、ｃＤＮＡの３’側の400bp 断片を得た。一方pUEX1(Amersham社製)をSmaIとPstIで消化し、CIP 処理した。このpUEX1 とｃＤＮＡの５’側の263bp 断片、ｃＤＮＡの３’側の400bp 断片のライゲーション反応を行い、組換えベクターpCI07 を得た。実施例１と同様にして塩基配列を決定し、この組換えベクターpCI07 には、ＨＣＶのN末端から数えて、1323番目から1533番目のアミノ酸配列をコードする塩基配列が含まれ、その塩基配列は配列番号６に示すとおりである。次に、組換えベクターpCI07 で宿主大腸菌HB101 を形質転換し、組換え大腸菌HB101 ［pCI07 ］を得た。組換え大腸菌HB101 ［pCI07 ］をＬＢ＋Amp培地で30℃で一晩培養し、最終濃度が15%となるようにグリセリンを添加して－80℃で凍結保存した。

（２－３）ＮＳ－３抗原の製造

組換え大腸菌HB101 ［pCI07 ］を培養し遺伝子発現を行うことにより、ＮＳ－３抗原はβ－ガラクトシダーゼとの融合ポリペプチドとして生産される。実施例１の（１－３）Ｃｏｒｅ抗原の製造と同様にして、組換え大腸菌HB101 ［pCI07］の培養、菌体の破砕、融合ポリペプチドの分離精製を行った。20リットルのＬＢ＋Amp培地にて培養し、1,000mg のＮＳ－３抗原が得られた。得られたＮＳ－３抗原について、ＳＤＳ－ＰＡＧＥにより分子量を調べそのアミノ酸配列より計算される分子量（141kd)と一致することを確認した。

【００３６】（実施例３）　ＮＳ－４抗原の製造

（３－１）組換えプラスミドpHCV10の作製

実施例１で得られたｃＤＮＡライブラリーをプラークハイブリダイゼーションによりスクリーニングした。まず大腸菌 Y1090を宿主とし、直径15cmのプレート10枚に、ｃＤＮＡライブラリーの組換えλgt11ファージ５×105相当を出現させた。得られたプラークを、ニトロセルロースに写し取り、ハイブリダイゼーションを行った。こうして、ＨＣＶ遺伝子断片をもつクローン６株を選択した。そして、このクローンからファージＤＮＡを回収し、次いでEcoRI で切断して、６種類のＨＣＶ遺伝子断片、H1 、H5 、H10、H13、H20、H21断片をアガロース電気泳動ゲルより回収した。このうち、優れた抗原活性に必須なアミノ酸配列をコードする塩基配列を含むH10断片について、該断片をプラスミドベクターpTZ19RのEcoRI 部位に挿入し、組換えプラスミドpHCV10を得た。

【００３７】組換えプラスミドpHCV10により形質転換された大腸菌は、E. coli HCV10 と表示し、通商産業省工業技術院微生物工業技術研究所に微工研菌寄第11834 号として寄託されている。

（３－２）大腸菌HB101 ［pCI10 ］の作製

pHCV10をAvaII で消化後、ＤＮＡポリメラーゼI Klenow fragmentにより末端を平滑化し、さらにBamHI で消化して583bp 断片を単離した。一方pUEX3 (Amersham社製) をSmaIで消化し、CIP 処理し、さらにBamHI で消化した。その後、電気泳動を行い目的の断片を分離した。これらをライゲーションし、組換えベクターpCI10 を作製した。実施例１と同様にして塩基配列を決定し、この組換えベクターpCI10 には、ＨＣＶのN末端から数えて、1605番目から1798番目のアミノ酸配列をコードする塩基配列が含まれ、その塩基配列は配列番号７に示すとおりでる。次に、組換えベクターpCI10 で宿主大腸菌HB101 を形質転換し、組換え大腸菌HB101 ［pCI10 ］を得た。組換え大腸菌HB101 ［pCI10 ］をＬＢ＋Amp培地で30℃で一晩培養し、最終濃度が15%となるようにグリセリンを添加して－80℃で凍結保存した。

（３－３）ＮＳ－４抗原の製造

組換え大腸菌HB101 ［pCI10 ］を培養し遺伝子発現を行うことにより、ＮＳ－４抗原はβ－ガラクトシダーゼとの融合ポリペプチドとして生産される。実施例１の（１－３）Ｃｏｒｅ抗原の製造と同様にして、組換え大腸菌HB101 ［pCI10］の培養、菌体の破砕、融合ポリペプチドの分離精製を行った。20リットルのＬＢ＋Amp培地にて培養し、720mg のＮＳ－４抗原が得られた。得られたＮＳ－４抗原について、ＳＤＳ－ＰＡＧＥにより分子量を調べそのアミノ酸配列より計算される分子量（141kd)と一致することを確認した。

【００３８】（実施例４）　ＮＳ－５抗原の製造

（４－１）組換えプラスミドpHCV14の作製

実施例２と同様にしてＲＴ－ＰＣＲ法により増幅した、ＨＣＶのＮＳ５領域の遺伝子産物のH14断片を、アガロースゲル(2%) で電気泳動し目的の長さのＤＮＡを回収した。なお、５’上流側プライマーの塩基配列は、（５’）ＣＧＧＧＣＡＴＧＡＣＣＡＣＴＧＡＣＡＡＣ（３’）、３’下流側プライマーの塩基配列は、（５’）ＣＣＧＣＣＴＣＴＡＧＧＡＣＧＣＴＴＴＴＧ（３’）である。ついでこれをKlenow fragment 酵素処理し、ＤＮＡの末端を平滑に揃え、更にT4 ポリヌクレオチドキナ

ーゼにより、５’末端をリン酸化した。これをプラスミドベクターpTZ19RのHincII部位に挿入し組換えプラスミドpHCV14を得た。組換えプラスミドpHCV14により形質転換された大腸菌は、E. coli HCV14 と表示し、通商産業省工業技術院微生物工業技術研究所に微工研菌寄第11838 号として寄託されている。

（４－２）大腸菌HB101 ［pCI14 ］の作製

pHCV14をPstI及びXbaIで消化後、blunting kitにより末端を平滑化し、484bpを含む断片を単離した。一方pUEX2 をSmaIで消化し、CIP 処理した。その後、電気泳動を行い目的の断片を分離した。これらをライゲーションし、組換えベクターpCI14 を作製した。実施例１と同様にして塩基配列を決定し、この組換えベクターpCI14 には、ＨＣＶのN末端から数えて、2111番目から2270番目のアミノ酸配列をコードする塩基配列が含まれ、その塩基配列は配列番号８に示すとおりである。次に、組換えベクターpCI14 で宿主大腸菌HB101 を形質転換し、組換え大腸菌HB101 ［pCI14 ］を得た。組換え大腸菌HB101 ［pCI14 ］をＬＢ＋Amp培地で30℃で一晩培養し、最終濃度が15％となるようにグリセリンを添加して−80℃で凍結保存した。

（４－３）ＮＳ－５抗原の製造

組換え大腸菌HB101 ［pCI14 ］を培養し遺伝子発現を行うことにより、ＮＳ－５抗原はβ－ガラクトシダーゼとの融合ポリペプチドとして生産される。実施例１の（１－３）Ｃｏｒｅ抗原の製造と同様にして、組換え大腸菌HB101 ［pCI14］の培養、菌体の破砕、融合ポリペプチドの分離精製を行った。20リットルのＬＢ＋Amp培地にて培養し、750mg のＮＳ－５抗原が得られた。得られたＮＳ－５抗原について、得られたＮＳ－４抗原について、ＳＤＳ－ＰＡＧＥにより分子量を調べそのアミノ酸配列より計算される分子量（135kd)と一致することを確認した。

【００３９】（実施例５）　ＨＣＶ抗原活性ポリペプチドを用いたＣ型肝炎診断用免疫学的凝集反応試薬

（加熱操作）　Ｃｏｒｅ抗原、ＮＳ－３抗原、ＮＳ－４抗原及びＮＳ－５抗原の４種のＨＣＶ抗原活性ポリペプチドを各々100μｇ／ｍｌずつをＰＢＳに等量分散して混合抗原溶液とする。該抗原混合溶液を35℃で30分間加熱して感作用抗原溶液とする

（感作）直径１．８μmのＨＤＰ（徳山曹達（株）製品）をＰＢＳで５（重量／重量）％になるように懸濁し、ＨＤＰ懸濁液とした。上記ＨＤＰ懸濁液１ｍｌと加熱操作を施した感作用抗原溶液１ｍｌを試験管内で混合して室温で１時間放置してＨＤＰ表面に４種類のＨＣＶ抗原活性ポリペプチドを疎水的に吸着させた（以下、この吸着操作を感作とも略記する）。

【００４０】（洗浄操作）その後、余剰のＨＣＶ抗原活性ポリペプチドを除去するために、上記混合液に2,500ｒｐｍ、５分間遠心分離を施し、遠心上清を除去した。その遠心沈澱物に洗浄のため、ＰＢＳ２ｍｌを添加、懸濁後2,500ｒｐｍ、５分間遠心後上清を除去し、３（vol/vol）％変性ウサギ血清含有ＰＢＳ（以下、Ａ液とも略記する）に0.5（w/vol）％になるように懸濁した。上記、ＨＣＶポリペプチドを吸着させたＨＤＰ（以下、感作粒子とも略記する）をＣ型肝炎診断用免疫学的凝集反応試薬（以下、Ｂ液とも略記する）とした。

【００４１】（測定操作）一方、検査に用いる検体をＡ液で２倍より倍数希釈して、8192倍まで希釈した。次に、検体の希釈液を９６穴マイクロタイタープレート（96well micro-titer-plate）に各々25μｌずつ１穴から12穴まで滴下した。ついで、上記で調製したＢ液を各穴25μｌを滴下した。滴下後、プレートミキサー（plate mixer)で振とうして30分間静置したのち、管底凝集像を観察した。管底凝集像のうち、抗原抗体反応が生じたために感作粒子がマイクロプレートの管底に広がったものを陽性像とし、抗原抗体反応が生じなかったために感作粒子がマイクロタイタープレートの管底に沈澱したのを陰性像とした。一般的にマイクロタイター試薬では検出感度は陽性像の観察される血清及び血漿の最高希釈倍率（以下、力価とも略記する）で表示するので以下、Ｃ型肝炎診断用免疫学的凝集反応試薬の感度は力価で表示する。なお、この力価は、健常者検体では低く、患者検体では高ければ高いほど良いとされる。

【００４２】（結果）次にＥＬＩＳＡ法試薬である市販品Ａと実施例５で調製したＣ型肝炎診断用免疫学的凝集反応試薬を比較した。検討には健常者検体５検体及び患者血清５検体を用いた。市販品Ａと本発明のＣ型肝炎診断用免疫学的凝集反応試薬は良好な相関を示したが、そのうち、１検体は本Ｃ型肝炎診断用免疫学的凝集反応試薬のみ、陽性を示した。なお、陽性と陰性との判断は32倍希釈より陽性像を示すものを陽性とした（表１参照）。

【００４３】健常者検体及び患者検体をさらに30検体を測定した（表２参照）。実施例５のＣ型肝炎診断用免疫学的凝集反応試薬で陽性且つ市販品Ａで陰性の判定の出る検体が２検体検出された。

【００４４】

【表１】

実施例５と市販品Ａの比較（１）

検体　No.	感度	
	実施例５	市販品Ａ（OD492）
1	>8192	++(>2.00)
2	8192	++(>2.00)
3	512	++(>2.00)
4	2048	++(>2.00)
5	64	-(0.114)
6	8	-(0.017)
7	16	-(0.042)
8	8	-(0.023)
9	16	-(0.006)
10	8	-(0.003)

【００４５】　【表２】

実施例５と市販品Ａの比較（２）

		実施例５	
		健常者検体	患者検体
市販品Ａ	陰性	14	2
	陽性	0	14

【００４６】比較例１　加熱処理を施さないＨＣＶ抗原活性ポリペプチドを用いたＣ型肝炎診断用免疫学的凝集反応試薬

（混合操作）Ｃｏｒｅ抗原、ＮＳ－３抗原、ＮＳ－４抗原及びＮＳ－５抗原の４種のＨＣＶ抗原活性ポリペプチドを各々100μｇ／ｍｌずつをＰＢＳに等量分散して混合抗原溶液して感作用抗原溶液とする。

【００４７】（感作）直径1.8μｍのＨＤＰ（徳山曹達（株）製品）をＰＢＳで５（w/w）％になるように懸濁し、ＨＤＰ懸濁液とした。上記感作用抗原液１ｍｌを試験管内に入れ、ＨＤＰ懸濁液と混合して室温で１時間放置してＨＤＰ表面に混合したＨＣＶ抗原活性ポリペプチドを感作した。

【００４８】（洗浄操作）実施例５と同様の操作で余剰のＨＣＶ抗原活性ポリペプチドを除去し、Ａ液にて粒子濃度0.5（w/vol）％となるように懸濁した。上記、感作粒子をＣ型肝炎診断用免疫学的凝集反応試薬（以下、Ｃ液とも略記する）とした。

（測定操作）実施例５と同様に検査に用いる検体をＡ液で２倍より倍数希釈して、8192倍まで希釈してＣ液、Ｄ液、Ｅ液及びＦ液を滴下後、管底凝集像を観察した。

【００４９】（結果）検討には実施例５のＣ型肝炎診断用免疫学的凝集反応試薬で力価が8192倍の検体（以下、患者検体１とも略記する）及び力価が８倍の検体（以下、健常者検体１とも略記する）を用いた。上記のＨＣＶ抗原活性ポリペプチドを感作したＣ型肝炎診断用免疫学的凝集反応試薬ともに実施例５で調製したＣ型肝炎診断用免疫学的凝集反応試薬より低い力価を示した（表３参照）。

【００５０】

【表３】

実施例５と比較例１の比較

	感　度	
	健常者検体１	患者検体１
実施例５	x8	x8192
比較例１	x8	x2048

【００５１】比較例２　１種類のＨＣＶ抗原活性ポリペプチドを用いたＣ型肝炎診断用免疫学的凝集反応試薬（加熱処理）Ｃｏｒｅ抗原、ＮＳ－３抗原、ＮＳ－４抗原及びＮＳ－５抗原の４種のＨＣＶ抗原活性ポリペプチドを各々100μｇ／ｍｌとなるようにＰＢＳに分散して、35℃で30分間、加熱処理して感作用Ｃｏｒｅ抗原液、感作用ＮＳ－３抗原液、感作用ＮＳ－４抗原液及び感作用ＮＳ－５抗原液をそれぞれ調製した。

【００５２】（感作）直径1.8μｍのＨＤＰ（徳山曹達（株）製品）をＰＢＳで５（w/w）％になるように懸濁し、ＨＤＰ懸濁液とした。上記感作用Ｃｏｒｅ抗原液、感作用ＮＳ－３抗原液、感作用ＮＳ－４抗原液及び感作用ＮＳ－５抗原液それぞれ１ｍｌを別々の試験管内に入れ、ＨＤＰ懸濁液と混合して室温で１時間放置してＨＤＰ表面にそれぞれのＨＣＶ抗原活性ポリペプチドを感作した。

【００５３】（洗浄操作）実施例５と同様の操作で余剰のＨＣＶ抗原活性ポリペプチドを除去し、Ａ液にて粒子濃度0.5（w/vol）％となるように懸濁した。上記、感作粒子をＣ型肝炎診断用免疫学的凝集反応試薬（以下、Ｄ、Ｅ、Ｆ及びＧ液とも略記する）とした。

（測定操作）実施例５と同様に検査に用いる検体をＡ液で２倍より倍数希釈して、8192倍まで希釈してＤ液、Ｅ液、Ｆ液及びＧ液を滴下後、管底凝集像を観察した。

【００５４】（結果）検討には患者検体１及び健常者検体１を用いた。上記のＨＣＶ抗原活性ポリペプチドを感作したＣ型肝炎診断用免疫学的凝集反応試薬ともに実施例５で調製したＣ型肝炎診断用免疫学的凝集反応試薬より低い力価を示した（表４参照）。

【００５５】

【表４】

実施例５と比較例２の比較

抗　原		感度	
		健常者検体１	患者検体１
実施例５	GCC-Core, NS-3 NS-4, NS-5	x8	x8192
比較例２	Core	x8	x256
	NS-3	x8	x128
	NS-4	x8	x64
	NS-5	x8	x128

【００５６】比較例３　２種類のＨＣＶ抗原活性ポリペプチドを用いたＣ型肝炎診断用免疫学的凝集反応試薬（加熱処理）Ｃｏｒｅ抗原とＮＳ－３抗原、Ｃｏｒｅ抗原とＮＳ－４抗原、Ｃｏｒｅ抗原とＮＳ－５抗原、ＮＳ－３抗原とＮＳ－４抗原、ＮＳ－３抗原とＮＳ－５抗原及びＮＳ－４抗原とＮＳ－５抗原の２種類ずつのＨＣＶ抗原活性ポリペプチドを各々50μｇ／ｍｌずつをＰＢＳに等量分散して35℃、30分間加熱処理をしてそれぞれ感作用抗原溶液１、２、３、４、５及び６とする。

【００５７】（感作）直径1.8μｍのＨＤＰ（徳山曹達（株）製品）をＰＢＳで５（ｗ／ｗ）％になるように懸濁し、ＨＤＰ懸濁液とした。上記ＨＤＰ懸濁液１ｍｌと感作用抗原溶液１ｍｌを試験管内で混合して室温で１時間放置してＨＤＰ表面に各々２種類のＨＣＶ抗原活性ポリペプチドを感作した。

【００５８】（洗浄操作）実施例５と同様の操作で余剰のＨＣＶ抗原活性ポリペプチドを除去し、Ａ液にて粒子濃度0.5（w/vol）％となるように懸濁した。上記、感作粒子をＣ型肝炎診断用免疫学的凝集反応試薬（以下、Ｈ、Ｉ、Ｊ、Ｋ、Ｌ及びＭ液とも略記する）とした。

【００５９】（測定操作）実施例５と同様に検査に用いる患者検体１及び健常者検体１をＡ液で２倍より倍数希釈し、8192倍まで希釈してＨ、Ｉ、Ｊ、Ｋ、Ｌ及びＭ液を滴下後、管底凝集像を観察した。

（結果）各々２種類のＨＣＶ抗原活性ポリペプチドを感作したＣ型肝炎診断用免疫学的凝集反応試薬ともに実施例５で調製したＣ型肝炎診断用免疫学的凝集反応試薬より低い力価を示した（表５参照）。

【００６０】

【表５】

実施例５比較例３の比較

抗原		感度	
		健常者検体１	患者検体１
実施例５	GCC-Core, NS-3 NS-4, NS-5	x8	x8192
比較例３	Core, NS-3	x8	x256
	Core, NS-4	x8	x128
	Core, NS-5	x8	x64
	NS-3, NS-4	x8	x84
	NS-3, NS-5	x8	x128
	NS-4, NS-5	x8	x64

【００６１】比較例４　３種類のＨＣＶ抗原活性ポリペプチドを用いたＣ型肝炎診断用免疫学的凝集反応試薬（加熱処理）Ｃｏｒｅ抗原とＮＳ－３抗原とＮＳ－４抗原、Ｃｏｒｅ抗原とＮＳ－３抗原とＮＳ－５抗原、Ｃｏｒｅ抗原とＮＳ－４抗原とＮＳ－５抗原及びＮＳ－３抗原とＮＳ－４抗原とＮＳ－５抗原の３種類ずつのＨＣＶ抗原活性ポリペプチドを各々３３μｇ／ｍｌずつをＰＢＳに等量分散して35℃、30分間の加熱処理を行い感作用抗原溶液７、８、９及び10とする。

【００６２】（感作）直径1.8μｍのＨＤＰ（徳山曹達（株）製品）をＰＢＳで５（w/w）％になるように懸濁し、ＨＤＰ懸濁液とした。上記ＨＤＰ懸濁液１ｍｌと各々の感作用抗原溶液１ｍｌを試験管内で混合して室温で１時間放置してＨＤＰ表面に各々３種類のＨＣＶ抗原活性ポリペプチドを感作した。

【００６３】（洗浄操作）実施例５と同様の操作で余剰のＨＣＶ抗原活性ポリペプチドを除去し、Ａ液にて粒子濃度0.5（w/vol）％となるように懸濁した。上記、感作粒子をＣ型肝炎診断用免疫学的凝集反応試薬（以下、Ｎ、Ｏ、Ｐ及びＱ液とも略記する）とした。

（測定操作）実施例５と同様に検査に用いる患者検体１及び健常者検体１をＡ液で２倍より倍数希釈し、8192倍まで希釈してＮ、０、Ｐ及びＱ液を滴下後、管底凝集像を観察した。

【００６４】（結果）各々３種類のＨＣＶ抗原活性ポリペプチドを感作したＣ型肝炎診断用免疫学的凝集反応試薬ともに実施例５で調製したＣ型肝炎診断用免疫学的凝集反応試薬より低い力価を示した（表６参照）。

【００６５】

【表６】

実施例５と比較例４の比較

抗　原		感度	
		健常者検体１	患者検体１
実施例５	GCC-Core, NS-3 NS-4, NS-5	x8	x8192
比較例４	Core, NS-3, NS-4	x8	x1024
	Core, NS-3, NS-5	x8	x512
	Core, NS-4, NS-5	x8	x128
	NS-3, NS-4, NS-5	x8	x64

【００６６】（実施例６）　羊赤血球担体を用いたＣ型肝炎診断用免疫学的凝集反応試薬

（羊赤血球の固定）羊赤血球100ｍｌ、オルセバ液100ｍｌを混合し、ガーゼ濾過ののち血球濃度測定後、生理食塩水で洗浄した。上記血球についてホルマリン固定を行った（Methods in Immunology and Immunochemistory, vol, pp33-34 (1977) (Williams, C, Hase編 Academic Press New Yorkによる））

（加熱処理）実施例５と同様にＣｏｒｅ抗原、ＮＳ－３抗原、ＮＳ－４抗原及びＮＳ－５抗原の４種のＨＣＶ抗原活性ポリペプチドをＰＢＳで各々100μｇ／ｍｌずつ等量混合した溶液を35℃、30分間加熱処理を行った（以下、Ｒ液とも略記する）。

【００６７】（感作）洗浄済み赤血球に３（vol／vol）％ホルマリン- 生理食塩水液を加え10℃で24時間撹拌したのち、さらに40（vol／vol）％ホルマリン- 生理食塩水液を追加して24時間撹拌した。生理食塩水で洗浄したのち、2.5（vol／vol）％となるように懸濁し、固定羊赤血球とした。

【００６８】上記Ｑ液１ｍｌとＰＢＳで５（w/w）％に

希釈した固定羊赤血球溶液1ｍｌを37℃で撹拌しながら、1時間、反応させた。この操作で4種のＨＣＶ抗原活性ポリペプチドを固定羊赤血球に感作した。

（洗浄操作）次いで実施例5と同様と洗浄操作を行い、余剰のＨＣＶ抗原活性ポリペプチドを除去し、Ａ液に粒子濃度0.5（w/vol）％となるように懸濁した。上記、感作粒子をＣ型肝炎診断用免疫学的凝集反応試薬（以下、Ｓ液とも略記する）とした。

【００６９】（測定操作）実施例5と同様に検査に用いる患者検体1及び健常者検体1をＡ液で2倍より倍数希釈し、8192倍まで希釈してＲ液を滴下後、管底凝集像を観察した。

（結果）実施例5と同様に、市販品Ａと実施例6で調製したＣ型肝炎診断用免疫学的凝集反応試薬を比較した。検討には健常者検体5検体及び患者血清5検体を用いた。市販品Ａと本Ｃ型肝炎診断用免疫学的凝集反応試薬は良好な相関を示したが、そのうち、1検体は本Ｃ型肝炎診断用免疫学的凝集反応試薬のみ、陽性を示した。なお、陽性と陰性との判断は３２倍希釈より陽性像を示すものを陽性とした（表7参照）。

【００７０】

【表7】

実施例6と市販品Ａの比較

検体　No.	感度	
	実施例6	市販品Ａ（OD492）
1	>8192	++(>2.00)
2	8192	++(>2.00)
3	256	++(>2.00)
4	2048	++(>2.00)
5	128	-(0.136)
6	16	-(0.027)
7	16	-(0.046)
8	8	-(0.043)
9	8	-(0.016)
10	8	-(0.003)

【００７１】比較例5　ＥＬＩＳＡ試薬と免疫学的凝集反応試薬の比較

（加熱処理）Ｃｏｒｅ抗原、ＮＳ－3抗原、ＮＳ－4抗原及びＮＳ－5抗原を100μｇ／ｍｌになるようにＰＢＳで分散したのち、35℃、30分間の加熱処理を行った。それぞれを加熱処理Ｃｏｒｅ抗原、加熱処理ＮＳ－3抗原、加熱処理ＮＳ－4抗原及び加熱処理ＮＳ－5抗原とする。また、Ｃｏｒｅ抗原、ＮＳ－3抗原、ＮＳ－4抗原及びＮＳ－5抗原を25μｇ／ｍｌずつ等量混合したのち、35℃、30分間の加熱処理を行い、加熱処理抗原溶液とする。

【００７２】（マイクロタイタープレートへの吸着）加熱処理Ｃｏｒｅ抗原、加熱処理ＮＳ－3抗原、加熱処理ＮＳ－4抗原及び加熱処理ＮＳ－5抗原をマイクロタイタープレート（ヌンク社製品）に1穴あたり50μｌずつ分注した。同様に該加熱処理抗原溶液をマイクロタイタープレートに1穴あたり50μｌずつ分注した。上記マイクロタイタープレートを37℃で1時間、吸着させた。吸着後、ＰＢＳ、200μｌで3回洗浄した。

【００７３】（ブロッキング操作）該マイクロタイタープレートに1％牛血アルブミン含有ＰＢＳ（以下、ＢＳＡ溶液とも略記する）を各穴に50μｌ分注して37℃で1時間、ブロッキングした。ブロッキング後、ＢＳＡ溶液を除去した。

（1次抗体反応）患者検体1及び健常者検体1各々10μｌをＢＳＡ溶液で10倍希釈したのち、各穴に分注して37℃で1時間、反応させた。

【００７４】（洗浄操作）1次抗体反応終了後、各検体の希釈液を除去した。0.5％トゥイーン80（tween80）含有ＰＢＳ溶液（以下、洗浄液とも略記する）200μｌで3回洗浄した。

（2次抗体反応）ＢＳＡ溶液で20,000倍に希釈したパーオキシダーゼ標識抗ヒトＩｇＧ（カペル社製品）を100μｌずつ各穴に分注して37℃で1時間反応させた。

【００７５】（洗浄操作）1次抗体反応終了後、各検体の希釈液を除去した。0.5％トゥイーン80（Tween80）含有ＰＢＳ溶液（以下、洗浄液とも略記する）200μｌで3回洗浄した。

（発色操作）過酸化水素水溶液（カペル社製品）とＡＢＴＳ溶液（カペル社製品）の等量混合液を100μｌを添加したのち、室温で30分間反応させた。反応後、10％ドデシル硫酸ナトリウム溶液を100μｌを添加して反応を停止し、該反応液の吸光度（波長414ｎｍ）を測定した。

【００７６】（結果）Ｃｏｒｅ抗原、ＮＳ－3抗原、ＮＳ－4抗原及びＮＳ－5抗原をそれぞれ吸着させた穴と4種類の該ＨＣＶ抗原活性ポリペプチドを混合した穴を比較したが、いつでも吸光度が同等で凝集反応試薬で見られたような高感度のＣ型肝炎診断用試薬は調製できなかった（表8参照）

【００７７】

【表8】

比較例５の結果

抗原	感度	
	健常者検体１ (OD414)	患者検体１ (OD414)
GCC-Core, NS-3 NS-4, NS-5	0.134	1.999
Core	0.111	1.922
NS-3	0.169	1.694
NS-4	0.158	1.825
NS-5	0.185	1.770

【００７８】
【配列表】
１．配列番号１
（１）配列の長さ：１６８
（２）配列の型：アミノ酸
（３）トポロジー：直鎖状
（４）配列の種類：タンパク質
（５）起源
生物名：ＨＣＶ（Ｃ型肝炎ウイルス）

（６）配列

Met Ser Thr Asn Pro Lys Pro Gln Arg Lys
5 10
Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln
15 20
Asp Val Lys Phe Pro Gly Gly Gly Gln Ile
25 30
Val Gly Gly Val Tyr Leu Leu Pro Arg Arg
35 40
Gly Pro Arg Leu Gly Val Arg Ala Thr Arg
45 50
Lys Thr Ser Glu Arg Ser Gln Pro Arg Gly
55 60
Arg Arg Gln Pro Ile Pro Lys Asp Arg Arg
65 70
Ser Thr Gly Lys Ser Trp Gly Lys Pro Gly
75 80
Tyr Pro Trp Pro Leu Tyr Gly Asn Glu Gly
85 90
Cys Gly Trp Ala Gly Trp Leu Leu Ser Pro
95 100
Arg Gly Ser Arg Pro Thr Trp Gly Pro Thr
105 110
Asp Pro Arg His Arg Ser Arg Asn Leu Gly
115 120
Lys Val Ile Asp Thr Ile Thr Cys Gly Phe
125 130
Ala Asp Leu Met Gly Tyr Ile Pro Val Val
135 140
Gly Ala Pro Val Gly Gly Val Ala Arg Ala
145 150
Leu Ala His Gly Val Arg Val Leu Glu Asp
155 160
Gly Val Asn Tyr Ala Thr Gly Asn
165

２．配列番号２
（１）配列の長さ：２１１
（２）配列の型：アミノ酸
（３）トポロジー：直鎖状

25

（４）配列の種類：タンパク質
（５）起源
生物名：ＨＣＶ（Ｃ型肝炎ウイルス）
（６）配列

Ser Thr Thr Ile Leu Gly Ile Gly Thr Val
5 10
Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg
15 20
Leu Val Val Leu Ala Thr Ala Thr Pro Pro
25 30
Gly Ser Ile Thr Val Pro His Pro Asn Ile
35 40
Glu Glu Val Ala Leu Ser Asn Thr Gly Glu
45 50
Ile Pro Phe Tyr Gly Lys Ala Ile Pro Ile
55 60
Glu Ala Ile Lys Gly Gly Arg His Leu Ile
65 70
Phe Cys His Ser Lys Lys Lys Cys Asp Glu
75 80
Leu Ala Ala Lys Leu Thr Gly Leu Gly Leu
85 90
Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp
95 100
Val Ser Val Ile Pro Thr Ser Gly Asp Val
105 110
Val Val Val Ala Thr Asp Ala Leu Met Thr
115 120
Gly Phe Thr Gly Asp Phe Asp Ser Val Ile
125 130
Asp Cys Asn Thr Cys Val Thr Gln Thr Val
135 140
Asp Cys Ser Leu Asp Pro Thr Phe Thr Ile
145 150
Glu Thr Thr Thr Val Pro Gln Asp Ala Val
155 160
Ser Arg Pro Gln Arg Arg Gly Arg Thr Gly
165 170
Arg Gly Arg Ser Gly Ile Tyr Arg Phe Val
175 180
Thr Pro Gly Glu Arg Pro Ser Gly Met Phe
185 190
Asp Ser Ser Val Leu Cys Glu Cys Tyr Asp
195 200
Ala Gly Cys Ala Trp Tyr Glu Leu Thr Pro
205 210
Ala

３．配列番号３
（１）配列の長さ：１９４
（２）配列の型：アミノ酸

26

（３）トポロジー：直鎖状
（４）配列の種類：タンパク質
（５）起源
生物名：ＨＣＶ（Ｃ型肝炎ウイルス）
（６）配列

Asp Gln Met Trp Lys Cys Leu Ile Arg Leu
5 10
Lys Pro Thr Leu His Gly Pro Thr Pro Leu
15 20
Leu Tyr Arg Leu Gly Ala Val Gln Asn Glu
25 30
Val Thr Leu Thr His Pro Ile Thr Lys Tyr
35 40
Ile Met Ala Cys Met Ser Ala Asp Leu Glu
45 50
Val Val Thr Ser Thr Trp Val Leu Val Gly
55 60
Gly Val Leu Ala Ala Leu Ala Ala Tyr Cys
65 70
Leu Thr Thr Gly Ser Val Val Ile Val Gly
75 80
Arg Ile Ile Leu Ser Gly Arg Pro Ala Val
85 90
Ile Pro Asp Arg Glu Val Leu Tyr Gln Glu
95 100
Phe Asp Glu Met Glu Glu Cys Ala Ser His
105 110
Leu Pro Tyr Ile Glu Gln Gly Met Gln Leu
115 120
Ala Glu Gln Phe Lys Gln Lys Ala Leu Gly
125 130
Leu Leu Gln Thr Ala Thr Lys Gln Ala Glu
135 140
Ala Ala Ala Pro Val Val Glu Ser Lys Trp
145 150
Arg Ala Leu Glu Val Phe Trp Ala Lys His
155 160
Met Trp Asn Phe Ile Ser Gly Ile Gln Tyr
165 170
Leu Ala Gly Leu Ser Thr Leu Pro Gly Asn
175 180
Pro Ala Ile Ala Ser Leu Met Ala Phe Thr
185 190
Ala Ser Ile Thr

４．配列番号４
（１）配列の長さ：１６０
（２）配列の型：アミノ酸
（３）トポロジー：直鎖状
（４）配列の種類：タンパク質
（５）起源

27

生物名：ＨＣＶ（Ｃ型肝炎ウイルス）

（６）配列

Lys Cys Pro Cys Gln Val Pro Ala Pro Glu
5 10
Phe Phe Thr Glu Val Asp Gly Val Arg Leu
15 20
His Arg Tyr Ala Pro Val Cys Lys Pro Leu
25 30
Leu Arg Glu Glu Val Val Phe Gln Val Gly
35 40
Leu Asn Gln Tyr Leu Val Gly Ser Gln Leu
45 50
Pro Cys Glu Pro Glu Pro Asp Val Ala Val
55 60
Leu Thr Ser Met Leu Thr Asp Pro Ser His
65 70
Ile Thr Ala Glu Met Ala Lys Arg Arg Leu
75 80
Ala Arg Gly Ser Pro Pro Ser Leu Ala Ser
85 90
Ser Ser Ala Ser Gln Leu Ser Ala Pro Ser
95 100
Leu Lys Ala Thr Cys Thr Thr His His Asp
105 110
Ser Pro Asp Ala Asp Leu Ile Glu Ala Asn
115 120
Leu Leu Trp Arg Gln Glu Met Gly Gly Asn
125 130
Ile Thr Arg Val Glu Ser Glu Asn Lys Val
135 140
Val Ile Leu Asp Ser Phe Asp Pro Ile Arg
145 150
Ala Val Glu Asp Glu Arg Glu Val Ser Val
155 160

５．配列番号５

（１）配列の長さ：５０４

（２）配列の型：核酸

（３）鎖の数：二本鎖

（４）トポロジー：直鎖状

（５）配列の種類：cDNA to genomic RNA

（６）起源

生物名：ＨＣＶ（Ｃ型肝炎ウィルス）

（７）配列の特徴：

特徴を表す記号：peptide

存在位置：1..504

特徴を決定した方法：Ｅ

28

（８）配列

ATG AGC ACA AAT CCT AAA CCT CAA AGA AAA 30
Met Ser Thr Asn Pro Lys Pro Gln Arg Lys
5 10
ACC AAA CGT AAC ACC AAC CGC CGC CCA CAG 60
Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln
15 20
GAC GTT AAG TTC CCG GGT GGC GGT CAG ATC 90
Asp Val Lys Phe Pro Gly Gly Gly Gln Ile
25 30
GTT GGC GGA GTT TAC CTG CTG CCG CGC AGG 120
Val Gly Gly Val Tyr Leu Leu Pro Arg Arg
35 40
GGC CCC AGG TTG GGT GTG CGC GCG ACA AGG 150
Gly Pro Arg Leu Gly Val Arg Ala Thr Arg
45 50
AAG ACT TCC GAG CGA TCC CAG CCG CGT GGA 180
Lys Thr Ser Glu Arg Ser Gln Pro Arg Gly
55 60
AGA CGC CAG CCC ATC CCG AAA GAT AGG CGC 210
Arg Arg Gln Pro Ile Pro Lys Asp Arg Arg
65 70
TCC ACC GGC AAG TCC TGG GGA AAG CCA GGA 240
Ser Thr Gly Lys Ser Trp Gly Lys Pro Gly
75 80
TAT CCT TGG CCT CTG TAT GGA AAC GAG GGT 270
Tyr Pro Trp Pro Leu Tyr Gly Asn Glu Gly
85 90
TGC GGC TGG GCA GGT TGG CTC CTG TCC CCC 300
Cys Gly Trp Ala Gly Trp Leu Leu Ser Pro
95 100
CGC GGA TCT CGT CCT ACT TGG GGC CCC ACT 330
Arg Gly Ser Arg Pro Thr Trp Gly Pro Thr
105 110
GAC CCC CGG CAC AGA TCG CGC AAT TTG GGC 360
Asp Pro Arg His Arg Ser Arg Asn Leu Gly
115 120
AAA GTC ATC GAC ACC ATT ACG TGT GGT TTT 390
Lys Val Ile Asp Thr Ile Thr Cys Gly Phe
125 130
GCC GAC CTC ATG GGG TAC ATC CCT GTC GTT 420
Ala Asp Leu Met Gly Tyr Ile Pro Val Val
135 140
GGC GCC CCG GTC GGA GGC GTC GCC AGA GCT 450
Gly Ala Pro Val Gly Gly Val Ala Arg Ala

```
                145                 150
CTG GCA CAC GGT GTT AGG GTC CTG GAA GAT  480
Leu Ala His Gly Val Arg Val Leu Glu Asp
                155                 160
GGG GTA AAT TAT GCA ACA GGG AAT          504
Gly Val Asn Tyr Ala Thr Gly Asn
                165
```

６．配列番号６

（１）配列の長さ：６３３

（２）配列の型：核酸

（３）鎖の数：二本鎖

（４）トポロジー：直鎖状

（５）配列の種類：cDNA to genomic RNA

（６）起源

生物名：ＨＣＶ（Ｃ型肝炎ウィルス）

（７）配列の特徴：

特徴を表す記号：peptide

存在位置：1..633

特徴を決定した方法：Ｅ

（８）配列

```
TCG ACT ACC ATC TTG GGC ATC GGC ACA GTC  30
Ser Thr Thr Ile Leu Gly Ile Gly Thr Val
                5                   10
CTG GAT CAG GCA GAG ACG GCT GGA GCG CGG  60
Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg
                15                  20
CTC GTC GTG CTC GCC ACC GCC ACG CCT CCG  90
Leu Val Val Leu Ala Thr Ala Thr Pro Pro
                25                  30
GGA TCG ATC ACC GTG CCA CAC CCC AAC ATC  120
Gly Ser Ile Thr Val Pro His Pro Asn Ile
                35                  40
GAG GAA GTG GCC CTG TCC AAC ACT GGG GAG  150
Glu Glu Val Ala Leu Ser Asn Thr Gly Glu
                45                  50
ATT CCC TTC TAT GGC AAA GCC ATC CCC ATT  180
Ile Pro Phe Tyr Gly Lys Ala Ile Pro Ile
                55                  60
GAG GCC ATC AAG GGG GGA AGG CAT CTC ATC  210
Glu Ala Ile Lys Gly Gly Arg His Leu Ile
                65                  70
TTC TGC CAT TCC AAG AAG AAG TGT GAC GAG  240
Phe Cys His Ser Lys Lys Lys Cys Asp Glu
                75                  80
CTC GCC GCA AAG CTG ACA GGC CTC GGA CTC  270
Leu Ala Ala Lys Leu Thr Gly Leu Gly Leu
                85                  90
AAT GCT GTA GCG TAT TAC AGG GGT CTC GAT  300
Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp
                95                  100
GTG TCC GTC ATA CCG ACT AGC GGA GAC GTC  330
Val Ser Val Ile Pro Thr Ser Gly Asp Val
                105                 110
GTT GTC GTG GCA ACA GAC GCT CTA ATG ACG  360
Val Val Val Ala Thr Asp Ala Leu Met Thr
                115                 120
GGT TTT ACC GGC GAC TTT GAC TCA GTG ATC  390
Gly Phe Thr Gly Asp Phe Asp Ser Val Ile
                125                 130
GAC TGC AAC ACA TGT GTC ACC CAG ACA GTC  420
Asp Cys Asn Thr Cys Val Thr Gln Thr Val
                135                 140
GAT TGC AGC TTG GAT CCC ACC TTC ACC ATT  450
Asp Cys Ser Leu Asp Pro Thr Phe Thr Ile
```

31

```
                145                 150
GAG ACG ACA ACC GTG CCC CAA GAC GCG GTG  480
Glu Thr Thr Thr Val Pro Gln Asp Ala Val
                155                 160
TCG CGT CCG CAG CGG CGA GGT AGG ACT GGC  510
Ser Arg Pro Gln Arg Arg Gly Arg Thr Gly
                165                 170
AGG GGC AGG AGT GGC ATC TAC AGG TTT GTG  540
Arg Gly Arg Ser Gly Ile Tyr Arg Phe Val
                175                 180
ACT CCA GGA GAA CGG CCC TCA GGC ATG TTC  570
Thr Pro Gly Glu Arg Pro Ser Gly Met Phe
                185                 190
GAC TCC TCG GTC CTG TGT GAG TGC TAT GAC  600
Asp Ser Ser Val Leu Cys Glu Cys Tyr Asp
                195                 200
GCA GGC TGC GCT TGG TAT GAG CTC ACG CCC  630
Ala Gly Cys Ala Trp Tyr Glu Leu Thr Pro
                205                 210
GCT                                     633
Ala
```

７．配列番号７

（１）配列の長さ：５８２

（２）配列の型：核酸

（３）鎖の数：二本鎖

（４）トポロジー：直鎖状

（５）配列の種類：cDNA to genomic RNA

（６）起源

生物名：ＨＣＶ（Ｃ型肝炎ウィルス）

（７）配列の特徴：

特徴を表す記号：peptide

存在位置：1..582

特徴を決定した方法：E

32

（８）配列

```
GAC CAA ATG TGG AAG TGT CTC ATA CGG CTA   30
Asp Gln Met Trp Lys Cys Leu Ile Arg Leu
                 5                  10
AAG CCC ACA CTG CAT GGG CCA ACG CCC CTG   60
Lys Pro Thr Leu His Gly Pro Thr Pro Leu
                15                  20
CTG TAC AGG CTA GGA GCC GTT CAA AAT GAG   90
Leu Tyr Arg Leu Gly Ala Val Gln Asn Glu
                25                  30
GTC ACT CTC ACA CAC CCC ATA ACC AAA TAC  120
Val Thr Leu Thr His Pro Ile Thr Lys Tyr
                35                  40
ATC ATG GCA TGC ATG TCG GCT GAC CTG GAG  150
Ile Met Ala Cys Met Ser Ala Asp Leu Glu
                45                  50
GTC GTC ACT AGC ACC TGG GTG CTA GTA GGC  180
Val Val Thr Ser Thr Trp Val Leu Val Gly
                55                  60
GGA GTC CTT GCG GCT CTG GCC GCG TAC TGC  210
Gly Val Leu Ala Ala Leu Ala Ala Tyr Cys
                65                  70
CTG ACG ACA GGC AGC GTG GTC ATT GTG GGC  240
Leu Thr Thr Gly Ser Val Val Ile Val Gly
                75                  80
AGG ATC ATC TTG TCC GGG AGG CCA GCT GTT  270
Arg Ile Ile Leu Ser Gly Arg Pro Ala Val
                85                  90
ATT CCC GAC AGG GAA GTC CTC TAC CAG GAG  300
Ile Pro Asp Arg Glu Val Leu Tyr Gln Glu
                95                 100
TTC GAT GAG ATG GAA GAG TGT GCT TCA CAC  330
Phe Asp Glu Met Glu Glu Cys Ala Ser His
               105                 110
CTC CCT TAC ATC GAG CAA GGA ATG CAG CTC  360
Leu Pro Tyr Ile Glu Gln Gly Met Gln Leu
               115                 120
GCC GAG CAA TTC AAA CAG AAG GCG CTC GGA  390
Ala Glu Gln Phe Lys Gln Lys Ala Leu Gly
               125                 130
TTG CTG CAA ACA GCC ACC AAG CAA GCG GAG  420
Leu Leu Gln Thr Ala Thr Lys Gln Ala Glu
               135                 140
GCT GCT GCT CCC GTG GTG GAG TCC AAG TGG  450
Ala Ala Ala Pro Val Val Glu Ser Lys Trp
```

```
                145                 150
CGA GCC CTT GAG GTC TTC TGG GCG AAA CAC  480
Arg Ala Leu Glu Val Phe Trp Ala Lys His
                155                 160
ATG TGG AAC TTC ATC AGC GGG ATA CAG TAC  510
Met Trp Asn Phe Ile Ser Gly Ile Gln Tyr
                165                 170
TTG GCA GGC CTA TCC ACT CTG CCT GGA AAC  540
Leu Ala Gly Leu Ser Thr Leu Pro Gly Asn
                175                 180
CCC GCG ATA GCA TCA TTG ATG GCT TTT ACA  570
Pro Ala Ile Ala Ser Leu Met Ala Phe Thr
                185                 190
GCC TCT ATC ACC                         582
Ala Ser Ile Thr
```

８．配列番号８

（１）配列の長さ：４８０

（２）配列の型：核酸

（３）鎖の数：二本鎖

（４）トポロジー：直鎖状

（５）配列の種類：cDNA to genomic RNA

（６）起源

生物名：ＨＣＶ（Ｃ型肝炎ウィルス）

（７）配列の特徴：

特徴を表す記号：peptide

存在位置：1..480

特徴を決定した方法：Ｅ

（８）配列

```
AAA TGC CCA TGC CAG GTT CCG GCC CCC GAA  30
Lys Cys Pro Cys Gln Val Pro Ala Pro Glu
                5                   10
TTT TTC ACG GAG GTG GAT GGA GTA CGG TTG  60
Phe Phe Thr Glu Val Asp Gly Val Arg Leu
                15                  20
CAC AGG TAT GCT CCG GTG TGC AAA CCT CTC  90
His Arg Tyr Ala Pro Val Cys Lys Pro Leu
                25                  30
CTA CGA GAG GAG GTC GTA TTC CAG GTC GGG 120
Leu Arg Glu Glu Val Val Phe Gln Val Gly
                35                  40
CTC AAC CAG TAC CTG GTC GGG TCA CAG CTC 150
Leu Asn Gln Tyr Leu Val Gly Ser Gln Leu
                45                  50
CCA TGT GAA CCC GAA CCG GAC GTA GCA GTG 180
Pro Cys Glu Pro Glu Pro Asp Val Ala Val
                55                  60
CTC ACT TCC ATG CTC ACC GAC CCC TCT CAT 210
Leu Thr Ser Met Leu Thr Asp Pro Ser His
                65                  70
ATT ACA GCA GAG ATG GCC AAG CGT AGG CTG 240
Ile Thr Ala Glu Met Ala Lys Arg Arg Leu
                75                  80
GCC AGG GGG TCT CCC CCC TCC TTG GCC AGC 270
Ala Arg Gly Ser Pro Pro Ser Leu Ala Ser
                85                  90
TCT TCA GCT AGC CAG TTG TCT GCG CCT TCT 300
Ser Ser Ala Ser Gln Leu Ser Ala Pro Ser
                95                 100
TTG AAG GCG ACA TGT ACT ACC CAT CAT GAC 330
Leu Lys Ala Thr Cys Thr Thr His His Asp
               105                 110
TCC CCG GAC GCT GAC CTC ATC GAG GCC AAC 360
Ser Pro Asp Ala Asp Leu Ile Glu Ala Asn
               115                 120
CTC CTG TGG CGG CAG GAG ATG GGC GGG AAC 390
Leu Leu Trp Arg Gln Glu Met Gly Gly Asn
               125                 130
ATC ACC CGA GTG GAG TCA GAA AAT AAG GTG 420
Ile Thr Arg Val Glu Ser Glu Asn Lys Val
               135                 140
GTA ATC CTG GAC TCT TTC GAT CCG ATT CGG 450
Val Ile Leu Asp Ser Phe Asp Pro Ile Arg
               145                 150
GCG GTG GAG GAT GAG AGG GAA GTA TCC GTT 480
Ala Val Glu Asp Glu Arg Glu Val Ser Val
               155                 160
```